

# PCT

## REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.
International Filing Date
Name of receiving Office and "PCT International Application"
Applicant's or agent's file reference (if desired) (12 characters maximum) 1107.85600

<b>Box No. I TITLE OF INVENTION</b> Mammalian Serine Racemase	
<b>Box No. II APPLICANT</b>	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)  THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE 111 Market Place Suite 906 Baltimore, Maryland 21202 United States of America	<input type="checkbox"/> This person is also inventor.  Telephone No. _____  Facsimile No. _____  Teleprinter No. _____ <div style="text-align: right;">N/A</div>
State (that is, country) of nationality: US	State (that is, country) of residence: US
This person is applicant <input type="checkbox"/> all designated States <input checked="" type="checkbox"/> all designated States except the United States of America for the purposes of: <input type="checkbox"/> the United States of America only <input type="checkbox"/> the States indicated in the Supplemental Box	
<b>Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)</b>	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)  SNYDER, Solomon H. c/o The Johns Hopkins University 111 Market Place, Suite 906 Baltimore, Maryland 21202 United States of America	This person is:  <input type="checkbox"/> applicant only  <input checked="" type="checkbox"/> applicant and inventor  <input type="checkbox"/> inventor only (if this check-box is marked, do not fill in below.)
State (that is, country) of nationality: US	State (that is, country) of residence: US
This person is applicant <input type="checkbox"/> all designated States <input type="checkbox"/> all designated States except the United States of America for the purposes of: <input checked="" type="checkbox"/> the United States of America only <input type="checkbox"/> the States indicated in the Supplemental Box	
<input type="checkbox"/> Further applicants and/or (further) inventors are indicated on a continuation sheet.	
<b>Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE</b>	
The person identified below is hereby/has been appointed to act on behalf <input checked="" type="checkbox"/> agent <input type="checkbox"/> common representative of the applicant(s) before the competent International Authorities as:	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)  KAGAN, Sarah A. BANNER & WITCOFF, LTD. 1001 G Street, N.W. Eleventh Floor Washington, D.C. 20001-4597 United States of America	Telephone No. (202) 508-9100  Facsimile No. (202) 508-9299  Teleprinter No. _____ <div style="text-align: right;">N/A</div>
<input type="checkbox"/> Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.	

## Continuation of Form PCT/RO/101 (continued from Sheet No. 1) FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

WOLOSKER, Herman  
c/o The Johns Hopkins University  
111 Market Place  
Suite 906  
Baltimore, Maryland 21202  
United States of America

This person is:

- ☐ applicant only  
☒ applicant and inventor  
☐ inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality: US

State (that is, country) of residence: US

This person is applicant ☐ all designated States ☐ all designated States except the United States of America  
for the purposes of: ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

SHETH, Kevin  
c/o The Johns Hopkins University  
111 Market Place  
Suite 906  
Baltimore, Maryland 21202  
United States of America

This person is:

- ☐ applicant only  
☒ applicant and inventor  
☐ inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality: US

State (that is, country) of residence: US

This person is applicant ☐ all designated States ☐ all designated States except the United States of America  
for the purposes of: ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

MASAAKI, Takahashi  
c/o The Johns Hopkins University  
111 Market Place  
Suite 906  
Baltimore, Maryland 21202  
United States of America

This person is:

- ☐ applicant only  
☒ applicant and inventor  
☐ inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality: US

State (that is, country) of residence: US

This person is applicant ☐ all designated States ☐ all designated States except the United States of America  
for the purposes of: ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

MOTHET, Jean-Pierre  
c/o The Johns Hopkins University  
111 Market Place  
Suite 906  
Baltimore, Maryland 21202  
United States of America

This person is:

- ☐ applicant only  
☒ applicant and inventor  
☐ inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality: US

State (that is, country) of residence: US

This person is applicant ☐ all designated States ☐ all designated States except the United States of America  
for the purposes of: ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on another continuation sheet

## Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTORS

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

BRADY, Jr. Roscoe O.  
c/o The Johns Hopkins University  
111 Market Place  
Suite 906  
Baltimore, Maryland 21202  
United States of America

This person is:

☐ applicant only☒ applicant and inventor☐ inventor only (if this check-box is marked, do not fill in below.)

State (that is, country) of nationality: US

State (that is, country) of residence: US

This person is applicant ☐ all designated States ☐ all designated States except the United States of America  
for the purposes of: ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

FERRIS, Christopher D.  
c/o The Johns Hopkins University  
111 Market Place  
Suite 906  
Baltimore, Maryland 21202  
United States of America

This person is:

☐ applicant only☒ applicant and inventor☐ inventor only (if this check-box is marked, do not fill in below.)

State (that is, country) of nationality: US

State (that is, country) of residence: US

This person is applicant ☐ all designated States ☐ all designated States except the United States of America  
for the purposes of: ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

This person is:

☐ applicant only☐ applicant and inventor☐ inventor only (if this check-box is marked, do not fill in below.)

State (that is, country) of nationality:

State (that is, country) of residence:

This person is applicant ☐ all designated States ☐ all designated States except the United States of America  
for the purposes of: ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

This person is:

☐ applicant only☐ applicant and inventor☐ inventor only (if this check-box is marked, do not fill in below.)

State (that is, country) of nationality:

State (that is, country) of residence:

This person is applicant ☐ all designated States ☐ all designated States except the United States of America  
for the purposes of: ☐ the United States of America only ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

**Box N . V DESIGNATION OF STATES**

The following designations are hereby made under Rule 4.9(a)(mark the applicable check-boxes; at least one must be marked):

**Regional Patent**

- ☒ **AP** **ARIPO Patent:** GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SL Sierra Leone, SZ Swaziland, TZ United Republic of Tanzania, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- ☒ **EA** **Eurasian Patent:** AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- ☒ **EP** **European Patent:** AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- ☒ **OA** **OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT (if other kind of protection or treatment desired, specify on dotted line)

**National Patent (if other kind of protection or treatment desired, specify on dotted line):**

- |   |  |
|---|--|
| <input checked="" type="checkbox"/> <b>AE</b> United Arab Emirates                  | <input checked="" type="checkbox"/> <b>LK</b> Sri Lanka  |
| <input checked="" type="checkbox"/> <b>AL</b> Albania                               | <input checked="" type="checkbox"/> <b>LR</b> Liberia  |
| <input checked="" type="checkbox"/> <b>AM</b> Armenia                               | <input checked="" type="checkbox"/> <b>LS</b> Lesotho  |
| <input checked="" type="checkbox"/> <b>AT</b> Austria                               | <input checked="" type="checkbox"/> <b>LT</b> Lithuania  |
| <input checked="" type="checkbox"/> <b>AU</b> Australia                             | <input checked="" type="checkbox"/> <b>LU</b> Luxembourg   |
| <input checked="" type="checkbox"/> <b>AZ</b> Azerbaijan                            | <input checked="" type="checkbox"/> <b>LV</b> Latvia   |
| <input checked="" type="checkbox"/> <b>BA</b> Bosnia and Herzegovina                | <input checked="" type="checkbox"/> <b>MA</b> Morocco  |
| <input checked="" type="checkbox"/> <b>BB</b> Barbados                              | <input checked="" type="checkbox"/> <b>MD</b> Republic of Moldova  |
| <input checked="" type="checkbox"/> <b>BG</b> Bulgaria                              | <input checked="" type="checkbox"/> <b>MG</b> Madagascar   |
| <input checked="" type="checkbox"/> <b>BR</b> Brazil                                | <input checked="" type="checkbox"/> <b>MK</b> The former Yugoslav Republic of Macedonia  |
| <input checked="" type="checkbox"/> <b>BY</b> Belarus                               | <input checked="" type="checkbox"/> <b>MN</b> Mongolia   |
| <input checked="" type="checkbox"/> <b>CA</b> Canada                                | <input checked="" type="checkbox"/> <b>MW</b> Malawi   |
| <input checked="" type="checkbox"/> <b>CH and LI</b> Switzerland and Liechtenstein  | <input checked="" type="checkbox"/> <b>MX</b> Mexico   |
| <input checked="" type="checkbox"/> <b>CN</b> China                                 | <input checked="" type="checkbox"/> <b>NO</b> Norway   |
| <input checked="" type="checkbox"/> <b>CR</b> Costa Rica                            | <input checked="" type="checkbox"/> <b>NZ</b> New Zealand  |
| <input checked="" type="checkbox"/> <b>CU</b> Cuba                                  | <input checked="" type="checkbox"/> <b>PL</b> Poland   |
| <input checked="" type="checkbox"/> <b>CZ</b> Czech Republic                        | <input checked="" type="checkbox"/> <b>PT</b> Portugal   |
| <input checked="" type="checkbox"/> <b>DE</b> Germany                               | <input checked="" type="checkbox"/> <b>RO</b> Romania  |
| <input checked="" type="checkbox"/> <b>DK</b> Denmark                               | <input checked="" type="checkbox"/> <b>RU</b> Russian Federation   |
| <input checked="" type="checkbox"/> <b>DM</b> Dominica                              | <input checked="" type="checkbox"/> <b>SD</b> Sudan  |
| <input checked="" type="checkbox"/> <b>EE</b> Estonia                               | <input checked="" type="checkbox"/> <b>SE</b> Sweden   |
| <input checked="" type="checkbox"/> <b>ES</b> Spain                                 | <input checked="" type="checkbox"/> <b>SG</b> Singapore  |
| <input checked="" type="checkbox"/> <b>FI</b> Finland                               | <input checked="" type="checkbox"/> <b>SI</b> Slovenia   |
| <input checked="" type="checkbox"/> <b>GB</b> United Kingdom                        | <input checked="" type="checkbox"/> <b>SK</b> Slovakia   |
| <input checked="" type="checkbox"/> <b>GD</b> Grenada                               | <input checked="" type="checkbox"/> <b>SL</b> Sierra Leone   |
| <input checked="" type="checkbox"/> <b>GE</b> Georgia                               | <input checked="" type="checkbox"/> <b>TJ</b> Tajikistan   |
| <input checked="" type="checkbox"/> <b>GH</b> Ghana                                 | <input checked="" type="checkbox"/> <b>TM</b> Turkmenistan   |
| <input checked="" type="checkbox"/> <b>GM</b> Gambia                                | <input checked="" type="checkbox"/> <b>TR</b> Turkey   |
| <input checked="" type="checkbox"/> <b>HR</b> Croatia                               | <input checked="" type="checkbox"/> <b>TT</b> Trinidad and Tobago  |
| <input checked="" type="checkbox"/> <b>HU</b> Hungary                               | <input checked="" type="checkbox"/> <b>TZ</b> United Republic of Tanzania  |
| <input checked="" type="checkbox"/> <b>ID</b> Indonesia                             | <input checked="" type="checkbox"/> <b>UA</b> Ukraine  |
| <input checked="" type="checkbox"/> <b>IL</b> Israel                                | <input checked="" type="checkbox"/> <b>UG</b> Uganda   |
| <input checked="" type="checkbox"/> <b>IN</b> India                                 | <input checked="" type="checkbox"/> <b>US</b> United States of America   |
| <input checked="" type="checkbox"/> <b>IS</b> Iceland                               | <input checked="" type="checkbox"/> <b>UZ</b> Uzbekistan   |
| <input checked="" type="checkbox"/> <b>JP</b> Japan                                 | <input checked="" type="checkbox"/> <b>VN</b> Viet Nam   |
| <input checked="" type="checkbox"/> <b>KE</b> Kenya                                 | <input checked="" type="checkbox"/> <b>YU</b> Yugoslavia   |
| <input checked="" type="checkbox"/> <b>KG</b> Kyrgyzstan                            | <input checked="" type="checkbox"/> <b>ZA</b> South Africa   |
| <input checked="" type="checkbox"/> <b>KP</b> Democratic People's Republic of Korea | <input checked="" type="checkbox"/> <b>ZW</b> Zimbabwe   |
| <input checked="" type="checkbox"/> <b>KR</b> Republic of Korea                     | Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet: |
| <input checked="" type="checkbox"/> <b>KZ</b> Kazakhstan                            | [ ]  |
| <input checked="" type="checkbox"/> <b>LC</b> Saint Lucia                           |  |

**Supplemental Box** If the Supplemental Box is not used, this sheet should not be included in the request.

1. If, in any of the Boxes, the space is insufficient to furnish all the information: in such case, write "Continuation of Box No. ..." [indicate the number of the Box] and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient, in particular:

(i) If more than two persons are involved as applicants and/or inventors and no "continuation sheet" is available: in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below;

(ii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication "the States Indicated in the Supplemental Box" is checked: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;

(iii) if, in Box No. II or in any of the sub-boxes of Box No. III, the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;

(iv) if, in addition to the agent(s) indicated in Box No. IV, there are further agents: in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;

(v) if, in Box No. V, the name of an State (or OAPI) is accompanied by the indication "patent of addition," or "certificate of addition," or if, in Box No. V, the name of the United States of America is accompanied by an indication "continuation" or "continuation-in-part": in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;

(vi) if, in Box No. VI, there are more than three earlier applications whose priority is claimed: in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI;

(vii) if, in Box No. VI, the earlier application is an ARIPO application: in such case, write "Continuation of Box No. VI", specify the number of the item corresponding to that earlier application and indicate at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed.

2. If, with regard to the precautionary designation statement contained in Box No. V, the applicant wishes to exclude any State(s) from the scope of that statement: in such case, write "Designation(s) excluded from precautionary designation statement" and indicate the name or two-letter code of each State so excluded.

3. If the applicant claims, in respect of any designated Office, the benefits of provisions of the national law concerning non-prejudicial disclosures or exceptions to lack of novelty: in such case write "Statement concerning non-prejudicial disclosures or exceptions to lack of novelty" and furnish that statement below.

Continuation of Box No. IV:

ALTHERR, Robert F.  
BANNER, Donald W.  
BANNER, Pamela I.  
BECKET, William W.  
FEDEROCHKO, Gary D.  
FISHER, William J.  
HONG, Patricia E.  
HOSCHEIT, Dale H.  
JACKSON, Thomas H.  
KAGAN, Sarah A.  
McKIE, Edward F. Jr.  
MEDLOCK, Nina L.  
NIEGOWSKI, James A.  
PETERSON, Thomas L.  
POTENZA, Joseph M.  
SKERPON, Joseph M.  
SCHAD, Steven P.  
WOLFFE, Franklin D.  
WOLFFE, Susan A.  
WRIGHT, Bradley C.

Continuation of Box No. V:

US Serial No.: 60/145,953 filed 28 July 1999 (28.07.99)  
US Serial No.: 60/144,839 filed 21 July 1999 (21.07.99)  
US Serial No.: 60/116,333 filed 19 January 1999 (19.01.99)

All members of the firm of BANNER & WITCOFF, LTD. at the address, telephone and telefacsimile numbers as indicated in Box No. IV.

**B x No. VI PRIORITY CLAIM**

[ ] Further priority claims as indicated in the Supplemental B x.

Filing date of earlier application (day/month/year)		Where earlier application is:		
Number of earlier application		national application: country	regional application: * regional Office	international application: receiving Office
item (1) 28 July 1999 (28.07.99)		60/145,953	US	
item (2) 21 July 1999 (21.07.99)		60/144,839	US	
item (3) 19 January 1999 (19.01.99)		60/116,333	US	

[X] The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) (only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office) identified above as item(s): (1), (2) and (3)

\* Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.

**B x No. VII INTERNATIONAL SEARCHING AUTHORITY**

Choice of International Searching Authority (ISA) (if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):  
ISA/EP

Request to use results of earlier search; reference to that search (if an earlier search has been carried out by or requested from the International Searching Authority):

Date (day/month/year) Number Country (or regional Office)

**B x No. VIII CHECK LIST; LANGUAGE OF FILING**

This international application contains the following number of sheets:

request : 7 sheets  
description (excluding sequence listing part) : 31 sheets  
claims : 3 sheets  
abstract : 1 sheets  
drawings : 7 sheets  
sequence listing part of description : 7 sheets  
Total number of sheets : 56 sheets

This international application is accompanied by the item(s) marked below:

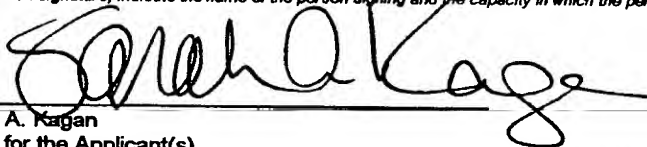
1. [X] fee calculation sheet (duplicate)
2. [ ] separate signed power of attorney
3. [ ] copy of general power of attorney; reference number, if any:
4. [ ] statement explaining lack of signature
5. [ ] priority document(s) identified in Box No. VI as item(s):
6. [ ] translation of international application into (language):
7. [ ] separate indications concerning deposited microorganism or other biological material
8. [ ] nucleotide and/or amino acid sequence listing in computer readable form
9. [X] other (specify): Transmittal

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: ENGLISH

**Box No. IX SIGNATURE OF APPLICANT OR AGENT**

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).



Sarah A. Kagan  
Agent for the Applicant(s)

For receiving Office use only

1. Date of actual receipt of the purported international application:	2. Drawings:  [ ] received:  [ ] not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:	
4. Date of timely receipt of the required corrections under PCT Article 11(2):	
5. International Searching Authority (if two or more are competent): ISA/EP	
6. [ ] Transmittal of search copy delayed until search fee is paid	

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

PCT

FEE CALCULATION SHEET  
Annex to the Request

Applicant's or agent's file reference 1107.85600		International application No.  Date stamp of the receiving Office	
Applicant THE JOHNS HOPKINS UNIVERSITY			
<b>CALCULATION OF PRESCRIBED FEES</b>			
1. TRANSMITTAL FEE	240	T	
2. SEARCH FEE	990	S	
International search to be carried out by EP <i>(If two or more International Searching Authorities are competent in relation to the international application, indicate the name of the Authority which is chosen to carry out the international search.)</i>			
3. INTERNATIONAL FEE			
<b>Basic Fee</b> The international application contains 56 sheets.  first 30 sheets			
	b <sub>1</sub>	427	
26 X 10.00 remaining sheets X additional amount =			
	b <sub>2</sub>	260	
Add amounts entered at b <sub>1</sub> and b <sub>2</sub> and enter total at B			
		687	B
<b>Designation Fees</b> The international application contains ALL designations. 8 x 92.00 number of designation fees x amount of designation fee payable (maximum 8) =			
		736	D
Add amounts entered at B and D and enter total at I			
		1573	I
<i>(Applicants from certain States are entitled to a reduction of 75% of the international fee. Where the applicant is (or all applicants are) so entitled, the total to be entered at I is 25% of the sum of the amounts entered at B and D.)</i>			
4. FEE FOR PRIORITY DOCUMENT (if applicable)		45	P
5. TOTAL FEES PAYABLE	<b>TOTAL: USD \$2848</b>		
Add amounts entered at T, S, I and P, and enter total in the TOTAL box			
<input type="checkbox"/> The designation fee is not paid at this time.			
<b>MODE OF PAYMENT</b>			
<input checked="" type="checkbox"/> authorization to charge deposit account (see below)			
<input type="checkbox"/> bank draft			
<input type="checkbox"/> coupons			
<input checked="" type="checkbox"/> cheque			
<input type="checkbox"/> cash			
<input type="checkbox"/> other (specify):			
<input type="checkbox"/> revenue stamps			
<input type="checkbox"/> postal money order			
<b>DEPOSIT ACCOUNT AUTHORIZATION</b> <i>(this mode of payment may not be available at all receiving Offices)</i> The RO/US <input type="checkbox"/> is hereby authorized to charge the total fees indicated above to my deposit account. <input checked="" type="checkbox"/> <i>(this check-box may be marked only if the conditions for deposit accounts of the receiving Office so permit)</i> is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account. <input checked="" type="checkbox"/> is hereby authorized to charge the fee for preparation and transmittal of the priority document to the International Bureau of WIPO to my deposit account.			
19-0733 Deposit Account Number		19 January 2000 Date (day/month/year)	
		Signature: Sarah A. Kagan, Reg. No.: 32,141	

The demand must be filed directly with the competent International Preliminary Examining Authority or, if two or more Authorities are competent, with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line below:  
**IPEA/ EP**

# **PCT DEMAND**

## **CHAPTER II**

under Article 31 of the Patent Cooperation Treaty:

The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).

For International Preliminary Examining Authority use only

Identification of IPEA	Date of receipt of DEMAND
------------------------	---------------------------

<b>Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION</b>		Applicant's or agent's file reference 1107.85600
International application No. PCT/US00/00938	International filing date (day/month/year) (18.01.00) 18 January 2000	(Earliest) Priority date (day/month/year) (19.01.99) 19 January 1999
Title of invention MAMMALIAN SERINE RACEMASE		
<b>Box No. II APPLICANT(S)</b>		
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)  The Johns Hopkins University 111 Market Place Suite 906 Baltimore, Maryland 21202 United States of America		Telephone No.:
		Facsimile No.:
		Teleprinter No.:
State (that is, country) of nationality: US		State (that is, country) of residence: US
Name and address: (Family name followed by given name; for legal entity, full official designation. The address must include postal code and name of country.)  SNYDER, Solomon H. c/o The Johns Hopkins University 111 Market Place, Suite 906 Baltimore, Maryland 21202 United States of America		
State (that is, country) of nationality: US		State (that is, country) of residence: US
Name and address: (Family name followed by given name; for legal entity, full official designation. The address must include postal code and name of country.)  WOLOSKER, Herman c/o The Johns Hopkins University 111 Market Place, Suite 906 Baltimore, Maryland 21202 United States of America		
State (that is, country) of nationality: US		State (that is, country) of residence: US
<input checked="" type="checkbox"/> Further applicants are indicated on a continuation sheet.		

International application No.  
PCT/US00/00938

**Continuation of Box No. II APPLICANT(S)**

*If none of the following sub-boxes is used, this sheet should not to be included in the demand.*

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

SHETH, Kevin  
c/o The Johns Hopkins University  
111 Market Place  
Suite 906  
Baltimore, Maryland 21202  
United States of America

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

MASAAKI, Takahashi  
c/o The Johns Hopkins University  
111 Market Place  
Suite 906  
Baltimore, Maryland 21202  
United States of America

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

MOTHET, Jean-Pierre  
c/o The Johns Hopkins University  
111 Market Place  
Suite 906  
Baltimore, Maryland 21202  
United States of America

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

BRADY, Jr. Roscoe O.  
c/o The Johns Hopkins University  
111 Market Place  
Suite 906  
Baltimore, Maryland 21202  
United States of America

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US



Further applicants are indicated on another continuation sheet.

International application No.  
PCT/US00/00938

**Continuation of Box No. II APPLICANT(S)**

*If none of the following sub-boxes is used, this sheet should not be included in the demand.*

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

FERRIS, Christopher D.  
c/o The Johns Hopkins University  
111 Market Place  
Suite 906  
Baltimore, Maryland 21202  
United States of America

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US



Further applicants are indicated on another continuation sheet.

International application No.  
PCT/US00/00938

**Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE**

The following person is ☒ agent ☐ common representative

and ☒ has been appointed earlier and represents the applicant(s) also for international preliminary examination.

☐ is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.

☐ is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

KAGAN, Sarah A.  
BANNER & WITCOFF, LTD.  
1001 G Street, N.W.  
Eleventh Floor  
Washington, D.C. 20001  
United States of America

Telephone No.:  
(202) 508-9100

Facsimile No.:  
(202) 508-9299

Teleprinter No.:  
N/A

☐ **Address for Correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

**Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION****Statement concerning amendments: \***

1. The applicant wishes the international preliminary examination to start on the basis of:

☒ the international application as originally filed

the description ☒ as originally filed

☐ as amended under Article 34

the claims ☒ as originally filed

☐ as amended under Article 19 (together with any accompanying statement)

☐ as amended under Article 34

the drawings ☒ as originally filed

☐ as amended under Article 34

2. ☐ The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.

3. ☐ The applicant wishes the start of the international preliminary examination to be postponed until the expiration of 20 months from the priority date unless the International Preliminary Examining Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired).*

\* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: ENGLISH

☒ which is the language in which the international application was filed.

☐ which is the language of a translation furnished for the purposes of international search.

☐ which is the language of publication of the international application.

☐ which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

**Box No. V ELECTION OF STATES**

The applicant hereby elects all eligible States *(that is, all States which have been designated and which are bound by Chapter II of the PCT)* excluding the following States which the applicant wishes not to elect: \_\_\_\_\_

International application No.  
PCT/US00/00938

**Box No. VI CHECK LIST**

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:

- |    |   |   |       |        |
|----|---|---|-------|--------|
| 1. | translation of international application                              | : | _____ | sheets |
| 2. | amendments under Article 34   | : | _____ | sheets |
| 3. | copy (or, where required, translation) of amendments under Article 19 | : | _____ | sheets |
| 4. | copy (or, where required, translation) of statement under Article 19  | : | _____ | sheets |
| 5. | letter  | : | _____ | sheets |
| 6. | other ( <i>specify</i> )  | : | _____ | sheets |

For International Preliminary  
Examining Authority use only

received                      not received

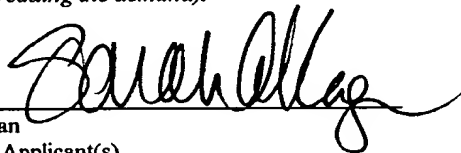
- |     |     |
|-----|-----|
| [ ] | [ ] |
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| [ ] | [ ] |
| [ ] | [ ] |
| [ ] | [ ] |
| [ ] | [ ] |

The demand is also accompanied by the item(s) marked below:

- |   |  |
|---|--|
| 1. [X] fee calculation sheet  | 4. [ ] statement explaining lack of signature                                  |
| 2. [ ] separate signed power of attorney                            | 5. [ ] nucleotide and/or amino acid sequence listing in computer readable form |
| 3. [ ] copy of general power of attorney, reference number, if any: | 6. [ ] other ( <i>specify</i> ):   |

**Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE**

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).



Sarah A. Kagan  
Agent for the Applicant(s)

For International Preliminary Examining Authority use only

- |    |   |
|----|---|
| 1. | Date of actual receipt of DEMAND  |
| 2. | Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b)  |
| 3. | [ ] The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply.<br>[ ] The applicant has been informed accordingly. |
| 4. | [ ] The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5.  |
| 5. | [ ] Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.                              |

For International Bureau use only

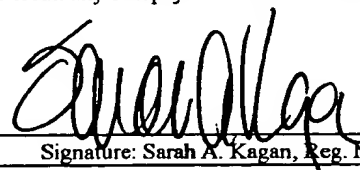
Demand received from IPEA on:

## PCT

## FEE CALCULATION SHEET

## Annex to the Demand for international preliminary examination

For International Preliminary Examining Authority use only

<p>International application No. <u>PCT/US00/00938</u></p> <hr/> <p>Applicant's or agent's file reference <u>1107.85600</u></p> <hr/> <p>Applicant <u>The Johns Hopkins University</u></p>	<p style="text-align: center;">Date stamp of the IPEA</p>
<p><b>Calculation of prescribed fees</b></p> <p>1. Preliminary examination fee ..... <u>EUR 1,533</u> [P]</p> <p>2. Handling fee (<i>Applicants from certain States are entitled to a reduction of 75% of the handling fee. Where the applicant is (or all applicants are) so entitled, the amount to be entered at H is 25% of the handling fee.</i>) ..... <u>EUR 147</u> [H]</p> <p>3. Total of prescribed fees Add the amounts entered at P and H and enter total in the TOTAL box ..... <u>EUR 1,680</u> TOTAL</p>	
<p><b>Mode of Payment</b></p> <p>[ ] authorization to charge deposit account with the IPEA (see below)      [ ] cash</p> <p>[ ] cheque      [ ] revenue stamps</p> <p>[ ] postal money order      [ ] coupons</p> <p>[ X ] bank draft      [ ] other (specify):</p>	
<p><b>Deposit Account Authorization</b> (<i>this mode of payment may not be available at all IPEAs</i>)</p> <p>The IPEA/ EP      [ ] is hereby authorized to charge the total fees indicated above to my deposit account.</p> <p>[ ] (<i>this check-box may be marked only if the conditions for deposit accounts of the IPEA so permit</i>) is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account.</p>	
<p style="text-align: center;"> <u>N/A</u>      <u>7 August 2000</u>        Deposit Account Number      Date (day/month/year)      Signature: Sarah A. Kagan, Reg. No.: 32,141 </p>	

## INTERNATIONAL SEARCH REPORT

National Application No

PCT/US 00/00938

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/61 C12N9/90 C12N15/85 C12N5/10 C12Q1/533

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TAKUMA UO ET AL.: "Occurrence of pyridoxal 5'-phosphate-dependent Serine racemase in silkworm, Bombyx mori" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 246, no. 1, 8 May 1998 (1998-05-08), pages 31-34, XP002135965 ORLANDO, FL US abstract page 32, right-hand column, paragraph 2 -page 33, left-hand column, paragraph 3 page 34, left-hand column, last paragraph ---	1-13
X	GB 2 048 266 A (MITSUITOATSU CHEMICALS) 10 December 1980 (1980-12-10)  page 2, line 16 - line 50 ----- -/--	1-6, 9-11, 24, 28, 32-38



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

## \* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&amp;" document member of the same patent family

Date of the actual completion of the international search

9 May 2000

Date of mailing of the international search report

23/05/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Montero Lopez, B

# INTERNATIONAL SEARCH REPORT

Patent Application No

PCT/US 00/00938

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DAVID S. DUNLOP ET AL.: "The origin and turnover of D-Serine in brain" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 235, no. 1, 9 June 1997 (1997-06-09), pages 26-30, XP002135966 ORLANDO, FL US abstract page 30, right-hand column, paragraph 2 - paragraph 3	1-13
P, X	HERMAN WOLOSKE ET AL.: "Serine racemase: a glial enzyme synthesizing D-Serine to regulate glutamate-N-methyl-D-aspartate neurotransmission" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 96, no. 23, 9 November 1999 (1999-11-09), pages 13409-13414, XP002135967 WASHINGTON US page 13410, left-hand column, paragraph 5 -page 13413, right-hand column, paragraph 2; figure 1	1-38
P, X	HERMAN WOLOSKE ET AL.: "Purification of Serine racemase: Bioynthesis of the neuromodulator D-Serine" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 96, no. 2, 19 January 1999 (1999-01-19), pages 721-725, XP002135968 WASHINGTON US page 722, right-hand column, paragraph 3- -page 725, left-hand column, last paragraph	1-13, 32-38

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 00/00938

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB 2048266 A	10-12-1980	JP 56134992 A	22-10-1981
		JP 1207308 C	11-05-1984
		JP 55148095 A	18-11-1980
		JP 57031438 B	05-07-1982
		JP 56005098 A	20-01-1981
		JP 61000070 B	06-01-1986
		AU 530435 B	14-07-1983
		AU 5797980 A	13-11-1980
		CA 1128443 A	27-07-1982
		CH 642947 A	15-05-1984
		DE 3017861 A	22-01-1981
		FR 2456140 A	05-12-1980
		IT 1145337 B	05-11-1986
		MX 6037 E	08-10-1984
		NL 8002603 A	11-11-1980
		US 4335209 A	15-06-1982

# PATENT COOPERATION TREATY

*Int*  
*SAX*  
*IDEX*  
*4/17/01*

From the  
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

<p>To:</p> <p>KAGAN, Sarah A. BANNER &amp; WITCOFF, LTD. 1001 G Street, N.W. Eleventh Floor Washington, DC 20001-4597 ETATS-UNIS D'AMERIQUE</p>		<p style="text-align: center; font-size: 2em; font-weight: bold;">PCT</p> <p style="text-align: center;">NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT (PCT Rule 71.1)</p>	
<p><i>01107.85600</i> <b>RECEIVED</b> <i>APR 16 2001</i> <i>BANNER &amp; WITCOFF LTD.</i> <i>National Phase</i> <i>7/19/01</i></p>		<p>Date of mailing (day/month/year)      09.04.2001</p>	
<p>Applicant's or agent's file reference 1107.85600</p>		<p><b>IMPORTANT NOTIFICATION</b></p>	
<p>International application No. PCT/US00/00938</p>	<p>International filing date (day/month/year) 18/01/2000</p>	<p>Priority date (day/month/year) 19/01/1999</p>	
<p>Applicant THE JOHNS HOPKINS UNIVERSITY</p>			

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

**4. REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

<p>Name and mailing address of the IPEA/</p> <p>----- European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016</p>	<p>Authorized officer</p> <p>Sinanovic, E</p> <p>Tel. +31 70 340-2672</p>
--	---



## TENT COOPERATION TREATY

## PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference <b>1107.85600</b>	<b>FOR FURTHER ACTION</b> See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. <b>PCT/US00/00938</b>	International filing date (day/month/year) <b>18/01/2000</b>	Priority date (day/month/year) <b>19/01/1999</b>
International Patent Classification (IPC) or national classification and IPC <b>C12N15/61</b>		
Applicant <b>THE JOHNS HOPKINS UNIVERSITY</b>		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.



2. This REPORT consists of a total of 7 sheets, including this cover sheet.

- ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☒ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand <b>07/08/2000</b>	Date of completion of this report <b>09.04.2001</b>
Name and mailing address of the international preliminary examining authority:  European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized officer <b>Montero Lopez, B</b> Telephone No. +31 70 340 3739 

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US00/00938

## I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).):*

### Description, pages:

1-31 as originally filed

### Claims, No.:

1-38 as originally filed

### Drawings, No.:

1-7 as originally filed

### Sequence listing part of the description, pages:

1-7, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US00/00938

- ☐ the description,      pages:
- ☐ the claims,      Nos.:
- ☐ the drawings,      sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

*(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

## V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

### 1. Statement

Novelty (N)	Yes:	Claims	4-8, 12-38
	No:	Claims	1-3 9-11
Inventive step (IS)	Yes:	Claims	4-6
	No:	Claims	1-3, 7-38
Industrial applicability (IA)	Yes:	Claims	1-38
	No:	Claims	

2. Citations and explanations  
**see separate sheet**

## VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:  
**see separate sheet**

## VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:  
**see separate sheet**

**Re Item V**

**Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

Reference is made to the following document:

D1: Biochemical and Biophysical Research Communications, 1998, vol. 246, No. 1, pages 31-34.

1. The underlying application refers to a Serine Racemase.
2. Document D1, which is considered to represent the most relevant state of the art, discloses (cf. page 32, col. right, par. 3 - page 33, col. left, par. 1) a silkworm Serine Racemase which has a specific activity in the conversion of L- to D-Serine of 1.1 units/mg. This corresponds, according to the definition of the assay for Serine Racemase disclosed in page 32, col. right, par. 2, to 0.06  $\mu\text{mol/mg/hour}$ .
  - 2.1. In addition to the feature relating to the specific activity of the enzyme, claims 1, and 9-11 attempt to define a product, an enzyme, according to the process to obtain it (of mammalian origin). However, the method of preparation does not impart any limitation to the product. A claim directed to a product according to the process to obtain the same is therefore construed as a claim to the product as such and the features relating to the process are entirely disregarded.
  - 2.2. For the reasons specified above, the Serine Racemase disclosed in D1, showing a specific activity of 0.06  $\mu\text{mol/mg/hour}$ , is prejudicial for the novelty of claims 1-3 and 9- 11, which therefore do not comply with the requirements of Article 33(2) PCT.
3. Claims 4-6 specify the activity of the enzyme being at least 1  $\mu\text{mol L-Serine/mg/hour}$ . No such enzyme has been disclosed in the state of the art and therefore claims 4-6 are novel and comply with the requirements of Article 33(2) PCT.

3.1. Document D1, which is considered to represent the most relevant state of the art, discloses (cf. page 32, col. right, par. 3 - page 33, col. left, par. 1) a silkworm Serine Racemase which has a specific activity in the conversion of L- to D-Serine of 0.06  $\mu\text{mol/mg/hour}$ . This activity is significantly lower than the activity of the enzymes claimed in claims 4-6. No hint exists in the state of the art which would allow the skilled person to obtain a serine racemase showing the claimed specific activity and therefore claims 4-6 are considered to involve an inventive step and to comply with the requirements of Article 33(3) PCT.

4. Claims 7, 8, 12, 13 further define the Serine Racemase by reference to its amino acid sequence. Since document D1 does not disclose the amino acid sequence of the enzyme, claims 7, 8, 12 and 13 are novel and meet the requirements of Article 33(3) PCT.

4.1. However, document D1 does specify the interest of determining the amino acid sequence of the enzyme (page 34, col. left, par. 3). The sequencing of a protein identified in the state of the art is a matter of routine for the skilled person which does not involve any inventive step. Claims 7, 8, 12 and 13, therefore do not involve an inventive step and do not comply with the requirements of Article 33(3) PCT.

5. Claims 14-31 relate to the polynucleotide encoding the Serine Racemase, as well as expression vectors and host cells comprising the same and their use for producing the enzyme. Such embodiments have not been disclosed in the state of the art and therefore claims 14-31 are novel according to Article 33(2) PCT.

5.1. However, document D1 suggests that the identification of the gene encoding the Serine Racemase may provide useful means for studying the role of D-Serine in mammalian brain (page 34, col. left, par. last). The skilled person would so be inevitably prompted to attempt the identification of the polynucleotide encoding the enzyme disclosed in D1, which he would put into practice by applying standard techniques in the art without the need of exercising any inventive step. Consequently, claims 14-31 do not involve an inventive step and do not meet the requirements of Article 33(3) PCT.

6. Claims 32-38 refer to the use of the Serine Racemase in a screening method to identify candidate therapeutic agents, not specifically disclosed in the state of the art and comply with the novelty requirements of Article 33(2) PCT.

6.1. However, such a screening process as disclosed in claims 32-38 constitutes a standard method in the art which the skilled person would apply without the need of exercising any inventive skill. Consequently, claims 32-38 do not involve an inventive step and do not meet the requirements of Article 33(3) PCT.

#### **Re Item VII**

##### **Certain defects in the international application**

1. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the document D1 is not mentioned in the description, nor is this document identified therein.

#### **Re Item VIII**

##### **Certain observations on the international application**

1. Claims 1, 13, 14 and 20 refer to "isolated" proteins and polynucleotides. Claims 14 and 20 refer as well to a "purified" polynucleotide. The applicant is kindly reminded that the degree of isolation or purification is not a technical feature of a preparation and the terms "isolated" and "purified" are therefore disregarded (Article 6 PCT).

2. Claims 1, 9-11, 13, 14, 20, 24, and 28-38 attempt to define a product, a protein, according to the process to obtain it (of mammalian, mouse, rat or human origin). However, the method of preparation does not impart any limitation to the product. A claim directed to a product according to the process to obtain the same is therefore construed as a claim to the product as such. In the present case, the product would be better defined in terms of its own structural features, such as its amino acid sequence (Article 6 PCT).

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

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International application No. PCT/US00/00938

3. Claims 1-6, 13 and 38, which define an enzyme with reference to its specific activity, do not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not clearly defined. The expression "specific activity" does not enable the skilled person to determine precisely the scope of the claim. The "specific activity" is defined in page 5 of the description, lines 21-25, with reference to Example 2 and other methods known in the art. It is therefore not clear in which conditions the specific activity is determined, which renders the scope of these claims unclear, contrary to Article 6 PCT.

4. Claims 1-6, 9-11, 14, 15, 21, 24, 28, and 32-38, 22-26, and 29 are defined merely in terms of an unclear functional feature (specific activity) and a process for preparation (mammalian origin). Such a definition renders the scope of the claims unclear and lacks the characterising features necessary for the adequate definition of the invention. The subject-matter of these claims is defined by merely stating an obviously desirable effect in view of the prior art, that is to provide a mammalian Serine Racemase (see page 34, col. left, last par. of D1). The present formulation of the claims in terms of functional features which merely state a desirable aim to be achieved and do not provide the necessary characterising features to solve the problem posed renders the scope of the claims unclear contrary to Article 6 PCT.

## PCT

NOTIFICATION OF THE RECORDING  
OF A CHANGE(PCT Rule 92bis.1 and  
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

KAGAN, Sarah, A.  
Banner & Witcoff, Ltd.  
11th floor  
1001 G Street, N.W.  
Washington, DC 20001-4597  
ETATS-UNIS D'AMERIQUENat'l Phase  
Ext 7-19-01

Date of mailing (day/month/year)

16 November 2000 (16.11.00)

Applicant's or agent's file reference

1107.85600

## IMPORTANT NOTIFICATION

International application No.

PCT/US00/00938

International filing date (day/month/year)

18 January 2000 (18.01.00)

## 1. The following indications appeared on record concerning:

☒ the applicant ☐ the inventor ☐ the agent ☐ the common representative

Name and Address

THE JOHNS HOPKINS UNIVERSITY  
SCHOOL OF MEDICINE  
Suite 906  
111 Market Place  
Baltimore, MD 21202  
United States of America

State of Nationality

US

State of Residence

US

Telephone No.

Facsimile No.

Teleprinter No.

## 2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person ☒ the name ☐ the address ☐ the nationality ☐ the residence

Name and Address

THE JOHNS HOPKINS UNIVERSITY  
Suite 906  
111 Market Place  
Baltimore, MD 21202  
United States of America

State of Nationality

US

State of Residence

US

Telephone No.

Facsimile No.

Teleprinter No.

## 3. Further observations, if necessary:

## 4. A copy of this notification has been sent to:

☒ the receiving Office ☐ the designated Offices concerned  
☐ the International Searching Authority ☒ the elected Offices concerned  
☒ the International Preliminary Examining Authority ☐ other:The International Bureau of WIPO  
34, chemin des Colombettes  
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

R. Chrem

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TRL.

From the INTERNATIONAL BUREAU

To:

KAGAN, Sarah, A.  
Banner & Witcoff, Ltd.  
11th floor  
1001 G Street, N.W.  
Washington, DC 20001-4597  
ETATS-UNIS D'AMERIQUE

SEP 20 2000

# INFORMATION CONCERNING ELECTED OFFICES NOTIFIED OF THEIR ELECTION

(PCT Rule 61.3)

Date of mailing (day/month/year)

12 September 2000 (12.09.00)

Applicant's or agent's file reference

1107.85600

## IMPORTANT INFORMATION

International application No.

PCT/US00/00938

International filing date (day/month/year)

18 January 2000 (18.01.00)

Priority date (day/month/year)

19 January 1999 (19.01.99)

Applicant

THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE et al

1. The applicant is hereby informed that the International Bureau has, according to Article 31(7), notified each of the following Offices of its election:

AP : GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW

EP : AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

National : AU, BG, CA, CN, CZ, DE, IL, JP, KP, KR, MN, NO, NZ, PL, RO, RU, SE, SK, US

2. The following Offices have waived the requirement for the notification of their election; the notification will be sent to them by the International Bureau only upon their request:

EA : AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

OA : BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

National : AE, AL, AM, AT, AZ, BA, BB, BR, BY, CH, CR, CU, DK, DM, EE, ES, FI, GB, GD, GE, GH,  
GM, HR, HU, ID, IN, IS, KE, KG, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MW, MX, PT, SD,  
SG, SI, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW

3. The applicant is reminded that he must enter the "national phase" **before the expiration of 30 months from the priority date** before each of the Offices listed above. This must be done by paying the national fee(s) and furnishing, if prescribed, a translation of the international application (Article 39(1)(a)), as well as, where applicable, by furnishing a translation of any annexes of the international preliminary examination report (Article 36(3)(b) and Rule 74.1).

Some offices have fixed time limits expiring later than the above-mentioned time limit. For detailed information about the applicable time limits and the acts to be performed upon entry into the national phase before a particular Office, see Volume II of the PCT Applicant's Guide.

The entry into the European regional phase is postponed **until 31 months from the priority date** for all States designated for the purposes of obtaining a European patent.

The International Bureau of WIPO  
34, chemin des Colombettes  
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer:

Juan Cruz

Telephone No. (41-22) 338.83.38

*sent to SAK/LNH*

INTERNET COOPERATION TREATY

RECEIVED

From the  
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

AUG 21 2000

PCT

To:

KAGAN, Sarah A.  
BANNER & WITCOFF, LTD.  
1001 G Street, N.W.  
Eleventh Floor  
Washington, DC 20001-4597  
ETATS-UNIS D'AMERIQUE

BANNER & WITCOFF LTD.

DOCKETED

COMMUNICATION IN CASES FOR WHICH  
NO OTHER FORM IS APPLICABLE

AUG 23 2000

*Response 9-16-00*

Date of mailing  
(day/month/year)

16.08.00

Applicant's or agent's file reference  
1107.85600

REPLY DUE

See paragraph 1 below

International application No.

PCT/US 00/00938

International filing date (day/month/year)

18/01/2000

Applicant

THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE

1. ☒ REPLY DUE within 1 , months/~~days~~ from the above date of mailing

☐ NO REPLY DUE

2. COMMUNICATION:

For completeness of the international records  
please clarify whether the official denomination of the  
first Applicant includes the part "School of Medicine"  
(cf. Request PCT/RO/101)

and also

whether the indication "Jr." should be part of the name of  
Applicant BRADY, R. O.

Thank you

Name and mailing address of the IPEA/



European Patent Office, P.B. 5818 Patentlaan 2  
NL-2280 HV Rijswijk - Netherlands  
Tel.: (+31-70) 340-2040, Tx. 31 651 epo nl  
Fax: (+31-70) 340-3016

Authorized officer

G.L.M. Kruydenberg  
070 - 3402277



*MB  
20/5-  
8/23/00*

# PATENT COOPERATION TREATY

From the  
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

## PCT

To:

KAGAN, Sarah A.  
BANNER & WITCOFF, LTD.  
1001 G Street, N.W.  
Eleventh Floor  
Washington, DC 20001-4597  
ETATS-UNIS D'AMERIQUE

### NOTIFICATION OF RECEIPT OF DEMAND BY COMPETENT INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

(PCT Rules 59.3(e) and 61.1(b), first sentence  
and Administrative Instructions, Section 601(a))

Date of mailing  
(day/month/year)

**1 6. 08. 00**

Applicant's or agent's file reference  
**1107.85600**

#### IMPORTANT NOTIFICATION

International application No.  
**PCT/US 00/ 00938**

International filing date (day/month/year)  
**18/01/2000**

Priority date (day/month/year)  
**19/01/1999**

Applicant

**THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE**

1. The applicant is hereby **notified** that this International Preliminary Examining Authority considers the following date as the date of receipt of the demand for international preliminary examination of the international application:

07/08/2000

2. This date of receipt is:

- ☒ the actual date of receipt of the demand by this Authority (Rule 61.1(b)).
- ☐ the actual date of receipt of the demand on behalf of this Authority (Rule 59.3(e)).
- ☐ the date on which this Authority has, in response to the invitation to correct defects in the demand (Form PCT/IPEA/404), received the required corrections.

3. ☐ **ATTENTION:** That date of receipt is **AFTER** the expiration of 19 months from the priority date. Consequently, the election(s) made in the demand does (do) not have the effect of postponing the entry into the national phase until 30 months from the priority date (or later in some Offices) (Article 39(1)). Therefore, the acts for entry into the national phase must be performed within 20 months from the priority date (or later in some Offices) (Article 22). For details, see the *PCT Applicant's Guide*, Volume II.

- ☐ (If applicable) This notification confirms the information given by telephone, facsimile transmission or in person on:

4. Only where paragraph 3 applies, a copy of this notification has been sent to the International Bureau.

Name and mailing address of the IPEA/

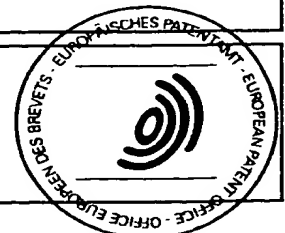


European Patent Office, P.B. 5818 Patentlaan 2  
NL-2280 HV Rijswijk - Netherlands  
Tel.: (+ 31-70) 340-2040, Tx. 31 651 epo nl  
Fax: (+ 31-70) 340-3016

Authorized officer

**KRUYDENBERG G L M**

Tel. (+ 31-70) 340-2277



**DOCKETED**

PATENT COOPERATION TREATY

WO 00/43526  
PCT/US00/00938*Sub 1/SAK/LMH*

AUG 8 2000

**PCT**

From the INTERNATIONAL BUREAU

To:

KAGAN, Sarah, A.  
Banner & Witcoff, Ltd.  
11th floor  
1001 G Street, N.W.  
Washington, DC 20001-4597  
ETATS-UNIS D'AMERIQUE**RECEIVED**

AUG 8 2000

BANNER &amp; WITCOFF LTD.

**NOTICE INFORMING THE APPLICANT OF THE  
COMMUNICATION OF THE INTERNATIONAL  
APPLICATION TO THE DESIGNATED OFFICES**

(PCT Rule 47.1(c), first sentence)

Date of mailing (day/month/year) 27 July 2000 (27.07.00)		
Applicant's or agent's file reference 1107.85600		<b>IMPORTANT NOTICE</b>
International application No. PCT/US00/00938	International filing date (day/month/year) 18 January 2000 (18.01.00)	
Priority date (day/month/year) 19 January 1999 (19.01.99)		
Applicant THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE et al		

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:

AU,JP,KP,KR,US

In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:

AE,AL,AM,AP,AT,AZ,BA,BB,BG,BR,BY,CA,CH,CN,CR,CU,CZ,DE,DK,DM,EA,EE,EP,ES,FI,GB,GD,  
GE,GH,GM,HR,HU,ID,IL,IN,IS,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,MN,MW,MX,NO,  
NZ,OA,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,UZ,VN,YU,ZA,ZW

The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on

27 July 2000 (27.07.00) under No. WO 00/43526

**REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)**

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, a demand for international preliminary examination must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

**REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))**

If the applicant wishes to proceed with the international application in the national phase, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer  J. Zahra
Facsimile No. (41-22) 740.14.35	Telephone No. (41-22) 338.83.38

PCT COOPERATION TREATY

*Out'l. / JAK/LMH*

From the INTERNATIONAL SEARCHING AUTHORITY

**PCT**

To:  
BANNER & WITCOFF, LTD.  
Attn. KAGAN, Sarah A.  
1001 G Street, N.W.  
Eleventh Floor  
Washington, DC 20001-4597  
UNITED STATES OF AMERICA

RECEIVED

MAY 24 2000

COMMUNICATION IN CASES FOR WHICH  
NO OTHER FORM IS APPLICABLE

REVIEWED

*OK*

Date of mailing  
(day/month/year) 15/05/2000

Applicant's or agent's file reference  
1107.85600

REPLY DUE  
See paragraph 1 below

International application No.  
PCT/US 00/ 00938

International filing date  
(day/month/year) 18/01/2000

Applicant

THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE

1. ☐ REPLY DUE within \_\_\_\_\_ ~~XXXX~~ days from the above date of mailing


☒ NO REPLY DUE

2. COMMUNICATION:

Please find enclosed a new copy of the Notification of Decision Concerning Request for Rectification (Form PCT/ISA/217).

The new substitute pages are stamped with the right Rule 91 stamp (English instead of French).

Kind regards,

Name and mailing address of the International Searching Authority  
 European Patent Office, P.B. 5818 Patentlaan 2  
NL-2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer  
Barbara Klaver

*BK*

25. 04. 2000

Our File No. 0707.85600**IN THE EUROPEAN PATENT OFFICE**

International Application No. <b>PCT/US00/00938</b>	International Filing Date <b>18 January 2000</b>	Priority Date Claimed <b>19 January 1999</b>
Title of Invention <b>MAMMALIAN SERINE RACEMASE</b>		
Applicant(s) <b>THE JOHNS HOPKINS UNIVERSITY</b>		

**VIA FEDERAL EXPRESS**

Zorka Bota, Authorized Officer  
EUROPEAN PATENT OFFICE  
P.B. 5818 Patentlaan 2  
NL-2280 HV Rijswijk  
NETHERLANDS

Attn.: ISA/EP

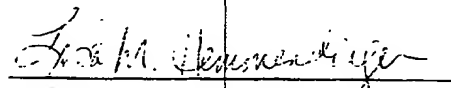
**STATEMENT ACCOMPANYING SEQUENCE LISTING**

Dear Sir:

The undersigned hereby states that the Sequence Listing submitted concurrently herewith does not include matter which goes beyond the content of the application as filed and that the information recorded on the diskette submitted concurrently herewith is identical to the written Sequence Listing.

Respectfully submitted,

Date: 21 April 2000

  
Lisa M. Hemmendinger  
Agent for the Applicants  
Reg. No. 42,653

BANNER & WITCOFF, LTD.  
1001 G STREET, N.W.  
ELEVENTH FLOOR  
WASHINGTON, D.C. 20001-4597  
UNITED STATES OF AMERICA  
(202) 508-9100

Our File No.: 1107.85600

**IN THE EUROPEAN PATENT OFFICE**

International Application No. <b>PCT/US00/00938</b>	International Filing Date <b>18 January 2000</b>	Priority Date Claimed <b>19 January 1999</b>
Title of Invention <b>MAMMALIAN SERINE RACEMASE</b>		
Applicant(s) <b>THE JOHNS HOPKINS UNIVERSITY</b>		

**VIA FEDERAL EXPRESS**

Zorka Bota, Authorized Officer  
EUROPEAN PATENT OFFICE  
P.B. 5818 Patentlaan 2  
NL-2280 HV Rijswijk  
NETHERLANDS

Attn.: ISA/EP

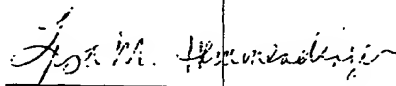
**TRANSMITTAL LETTER**

Dear Sir:

In response to the Invitation, Form PCT/ISA/225, mailed from the ISA/EP on 03 April 2000, a Sequence Listing in paper (2 sheets) and machine readable form and a statement to accompany the Sequence Listing are enclosed for the above-identified international application.

Respectfully submitted,

Date: 21 April 2000



Lisa M. Hemmendinger  
Agents for the Applicants  
Reg. No. 42,653

BANNER & WITCOFF, LTD.  
1001 G STREET, N.W.  
ELEVENTH FLOOR  
WASHINGTON, D.C. 20001-4597  
UNITED STATES OF AMERICA  
(202) 508-9100

INTERNATIONAL SEARCHING AUTHORITY  
THE EUROPEAN PATENT OFFICE

In the International Application of:

The Johns Hopkins University

Serial No. PCT/US00/00938

Filed: 18 January 2000

)  
)  
)  
)  
)  
)

Atty. Docket No. 1107.85600

For: MAMMALIAN SERINE RACEMASE

**REQUEST TO RECTIFY OBVIOUS ERRORS UNDER PCT RULE 91.1**

International Searching Authority  
European Patent Office

Sir or Madam:

Applicant respectfully requests permission to correct three obvious errors in the PCT application referenced above.

The first error is at page 4, line 27 in the Brief Description of FIG. 7. In the application as filed, FIG. 7 is described as depicting the "complete coding sequence of mouse serine racemase (SEQ ID NO:9) . . . ." SEQ ID NO:9, however, is not the coding sequence of mouse serine racemase, but is clearly labeled as a *Homo sapiens* DNA sequence (see page 40 of the originally filed sequence listing). Moreover, as can be seen from a visual comparison of the coding sequence in FIG. 7 and the DNA sequence shown in SEQ ID NO:9, these two sequences are not the same.

The second error is in the sequence listing originally filed. This sequence listing did not contain the coding sequence of mouse serine racemase shown in FIG. 7 and referred to at page 4, line 27.

The third obvious error is in the title of the application listed on the first page of the Sequence Listing. The title on that page reads "Mammalian Serine Protease," rather than "Mammalian Serine Racemase."

Applicant would like to correct these obvious errors by providing a substitute sequence listing and by providing a substitute page 4. The substitute sequence listing includes the coding sequence of mouse serine racemase shown in FIG. 7 as SEQ ID NO:11. Inclusion of this sequence as SEQ ID NO:11 is obvious because the coding sequence of mouse serine racemase is provided in FIG. 7, as stated on page 4. The title of the application also is corrected in the substitute sequence listing, from "Mammalian Serine Protease" to "Mammalian Serine Racemase." This correction, too, is obvious, as the subject matter to which the application is directed is clearly mammalian serine racemase, not serine protease.

Substitute page 4, line 27 refers to SEQ ID NO:11 as the coding sequence of mouse serine racemase. This correction is obvious because there is only one nucleotide sequence shown in FIG. 7 and that particular nucleotide sequence is not otherwise depicted in the originally filed sequence listing.

Applicant respectfully requests that the International Searching Authority authorize rectification of the errors described above.

Respectfully submitted,

Dated: 4-21-00

By: \_\_\_\_\_

*Lisa M. Hemmendinger*

Lisa M. Hemmendinger  
Registration No. 42,653

Banner & Witcoff, Ltd.  
1001 G Street, N.W., Suite 1100  
Washington, D.C. 20001-4597  
202-508-9100

chemiluminescence and HPLC assay. The experiment was replicated three times using different preparations with similar results.

**Figure 3.** Kinetic parameters of racemization reaction. Initial rate of racemase activity was measured at 37 °C in a medium containing 50 mM Tris-HCl (pH 8.0), 35 µg/ml purified enzyme, 1 mM EDTA, 2 mM DTT, 15 µM pyridoxal 5'-phosphate, and different concentrations of either L- or D- serine. The reaction was stopped after 2 hours, when less than 10% of the substrate was consumed. Values for  $K_m$  and  $V_{max}$  were calculated using the Michaelis-Menten equation. The values are representative of three experiments with different enzyme preparations.

**Figure 4.** Inhibition of serine racemase by PLP inhibitors and sulfhydryl oxidation. **Figure 4A.** Enzyme activity was monitored at 37 °C in a medium containing 50 mM Tris-HCl (pH 8.0), 20 mM L-serine, 40-100 µg/ml purified enzyme, 1 mM EDTA, 2 mM DTT, 10 µM pyridoxal 5'-phosphate and different concentrations of either aminooxyacetic acid (○) or hydroxylamine (●). **Figure 4B.** Reaction medium and conditions were as described in A, except that DTT was omitted from the last step of the enzyme preparation. The enzyme was preincubated for 10 minutes in the presence of different concentrations of oxidized glutathione (GSSG).

**Figure 5.** Absorption spectra of purified serine racemase. Purified enzyme (70 µg/ml) was preincubated for 10 minutes in medium containing 10 mM KPi (pH 7.2), 2 mM DTT, 1 mM EDTA, 10 µM PLP, either in the absence (Control) or in the presence of 1 mM aminooxyacetic acid (AOAA). The distinct peaks of absorbance at 420 and 340 nm were not observed in the presence of buffer alone or when bovine serum albumin was used instead of serine racemase.

**Figure 6.** Production of D-serine in culture medium of cells transfected with cDNA encoding full-length serine racemase (left) and in the transfected cells (right).

**Figure 7.** Complete coding sequence of mouse serine racemase (SEQ ID NO: 11) and its translated amino acid sequence (SEQ ID NO:8).

## **DETAILED DESCRIPTION OF THE INVENTION**

The inventors' isolation of serine racemase represents the first purification of an enzyme which converts L-serine to D-serine. Bacteria contain substantial levels of D-serine and many other D-amino acids. Though a number of amino acid

SEQUENCE LISTING

<110> Wolosker, Herman  
Takashashi, Maasaki  
Mothet, Jean-Pierre  
Ferris, Christopher  
Snyder, Solomon

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## PARENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

NOTIFICATION CONCERNING  
SUBMISSION OR TRANSMITTAL  
OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To:

KAGAN, Sarah, A.  
Banner & Witcoff, Ltd.  
11th floor  
1001 G Street, N.W.  
Washington, DC 20001-4597  
ETATS-UNIS D'AMERIQUEDate of mailing (day/month/year)  
27 March 2000 (27.03.00)Applicant's or agent's file reference  
1107.85600

## IMPORTANT NOTIFICATION

International application No.  
PCT/US00/00938International filing date (day/month/year)  
18 January 2000 (18.01.00)International publication date (day/month/year)  
Not yet publishedPriority date (day/month/year)  
19 January 1999 (19.01.99)

Applicant

THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE et al

1. The applicant is hereby notified of the date of receipt (except where the letters "NR" appear in the right-hand column) by the International Bureau of the priority document(s) relating to the earlier application(s) indicated below. Unless otherwise indicated by an asterisk appearing next to a date of receipt, or by the letters "NR", in the right-hand column, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).
2. This updates and replaces any previously issued notification concerning submission or transmittal of priority documents.
3. An asterisk(\*) appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b). In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.
4. The letters "NR" appearing in the right-hand column denote a priority document which was not received by the International Bureau or which the applicant did not request the receiving Office to prepare and transmit to the International Bureau, as provided by Rule 17.1(a) or (b), respectively. In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

<u>Priority date</u>	<u>Priority application No.</u>	<u>Country or regional Office or PCT receiving Office</u>	<u>Date of receipt of priority document</u>
19 Janu 1999 (19.01.99)	60/116,333	US	14 Marc 2000 (14.03.00)
21 July 1999 (21.07.99)	60/144,839	US	14 Marc 2000 (14.03.00)
28 July 1999 (28.07.99)	60/145,953	US	14 Marc 2000 (14.03.00)

APR 10 2000

The International Bureau of WIPO  
34, chemin des Colombettes  
1211 Geneva 20, Switzerland

Authorized officer

S. Mafla

Facsimile No. (41-22) 740.14.35

Telephone No. (41-22) 338.83.38

## PATENT COOPERATION TREATY

RECEIVED

MAY 18 2000

From the INTERNATIONAL SEARCHING AUTHORITY

REVIEWED

PCT

BANNER &amp; WITCOFF, LTD.

To:  
BANNER & WITCOFF, LTD.  
Attn. KAGAN, Sarah A.  
1001 G Street, N.W.  
Eleventh Floor  
Washington, DC 20001-4597  
UNITED STATES OF AMERICA

*Correct Defect*  
*6-17-00*

NOTIFICATION OF DECISION CONCERNING  
REQUEST FOR RECTIFICATION

(PCT Rule 91.1(f))

Date of mailing (day/month/year)	12/05/2000
Applicant's or agent's file reference 1107.85600	REPLY DUE NONE However, see last paragraph below
International application N°. PCT/US 00/ 00938	International filing date (day/month/year) 18/01/2000
Applicant THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE	

The applicant is hereby notified that this International Searching Authority has considered the request for rectification of obvious errors in the international application/in other papers submitted by the applicant to this Authority, and that it has decided:


1. ☒ to authorize the rectification:
- ☒ as requested by the applicant.
- ☐ to the extent set forth below\*:

2. ☐ to refuse to authorize the rectification or part of it for the following reasons\*:

A copy of this notification, together with a copy of the applicant's request for rectification, has been sent to the receiving Office and to the International Bureau.

\* If the authorization of the rectification has been refused in whole or in part, the applicant may request the International Bureau, before the technical preparations for international publication have been completed and subject to the payment of a fee, to publish the request for rectification together with the international application. See Rule 91.1(f), third and fourth sentences, and, for the amount of the fee, see the PCT Applicant's Guide, Volume I/A, Annex B2(IB).

Name and mailing address of the International Searching Authority

 European Patent Office, P.B. 5818 Patentlaan 2  
NL-2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Barbara Klaver *BK*

25.04.2000

Our File No. 07/07.85600

**IN THE EUROPEAN PATENT OFFICE**

International Application No. <b>PCT/US00/00938</b>	International Filing Date <b>18 January 2000</b>	Priority Date Claimed <b>19 January 1999</b>
Title of Invention <b>MAMMALIAN SERINE RACEMASE</b>		
Applicant(s) <b>THE JOHNS HOPKINS UNIVERSITY</b>		

**VIA FEDERAL EXPRESS**

Zorka Bota, Authorized Officer  
EUROPEAN PATENT OFFICE  
P.B. 5818 Patentlaan 2  
NL-2280 HV Rijswijk  
NETHERLANDS

Attn.: ISA/EP

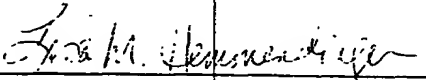
**STATEMENT ACCOMPANYING SEQUENCE LISTING**

Dear Sir:

The undersigned hereby states that the Sequence Listing submitted concurrently herewith does not include matter which goes beyond the content of the application as filed and that the information recorded on the diskette submitted concurrently herewith is identical to the written Sequence Listing.

Respectfully submitted,

Date: 21 April 2000

  
\_\_\_\_\_  
Lisa M. Hemmendinger  
Agent for the Applicants  
Reg. No. 42,653

BANNER & WITCOFF, LTD.  
1001 G STREET, N.W.  
ELEVENTH FLOOR  
WASHINGTON, D.C. 20001-4597  
UNITED STATES OF AMERICA  
(202) 508-9100

Our File No.: 1107.85600

**IN THE EUROPEAN PATENT OFFICE**

International Application No. <b>PCT/US00/00938</b>	International Filing Date <b>18 January 2000</b>	Priority Date Claimed <b>19 January 1999</b>
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Title of Invention <b>MAMMALIAN SERINE RACEMASE</b>
--

Applicant(s) <b>THE JOHNS HOPKINS UNIVERSITY</b>
---

**VIA FEDERAL EXPRESS**

Zorka Bota, Authorized Officer  
EUROPEAN PATENT OFFICE  
P.B. 5818 Patentlaan 2  
NL-2280 HV Rijswijk  
NETHERLANDS

Attn.: ISA/EP

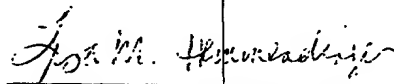
**TRANSMITTAL LETTER**

Dear Sir:

In response to the Invitation, Form PCT/ISA/225, mailed from the ISA/EP on 03 April 2000, a Sequence Listing in paper (2 sheets) and machine readable form and a statement to accompany the Sequence Listing are enclosed for the above-identified international application.

Respectfully submitted,

Date: 21 April 2000



Lisa M. Hemmendinger  
Agents for the Applicants  
Reg. No. 42,653

BANNER & WITCOFF, LTD.  
1001 G STREET, N.W.  
ELEVENTH FLOOR  
WASHINGTON, D.C. 20001-4597  
UNITED STATES OF AMERICA  
(202) 508-9100

**INTERNATIONAL SEARCHING AUTHORITY  
THE EUROPEAN PATENT OFFICE**

In the International Application of:

The Johns Hopkins University

Serial No. PCT/US00/00938

Filed: 18 January 2000

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)  
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)  
)  
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Atty. Docket No. 1107.85600

For: **MAMMALIAN SERINE RACEMASE**

**REQUEST TO RECTIFY OBVIOUS ERRORS UNDER PCT RULE 91.1**

International Searching Authority  
European Patent Office

Sir or Madam:

Applicant respectfully requests permission to correct three obvious errors in the PCT application referenced above.

The first error is at page 4, line 27 in the Brief Description of FIG. 7. In the application as filed, FIG. 7 is described as depicting the "complete coding sequence of mouse serine racemase (SEQ ID NO:9) . . . ." SEQ ID NO:9, however, is not the coding sequence of mouse serine racemase, but is clearly labeled as a *Homo sapiens* DNA sequence (see page 40 of the originally filed sequence listing). Moreover, as can be seen from a visual comparison of the coding sequence in FIG. 7 and the DNA sequence shown in SEQ ID NO:9, these two sequences are not the same.

The second error is in the sequence listing originally filed. This sequence listing did not contain the coding sequence of mouse serine racemase shown in FIG. 7 and referred to at page 4, line 27.

The third obvious error is in the title of the application listed on the first page of the Sequence Listing. The title on that page reads "Mammalian Serine Protease," rather than "Mammalian Serine Racemase."

Applicant would like to correct these obvious errors by providing a substitute sequence listing and by providing a substitute page 4. The substitute sequence listing includes the coding sequence of mouse serine racemase shown in FIG. 7 as SEQ ID NO:11. Inclusion of this sequence as SEQ ID NO:11 is obvious because the coding sequence of mouse serine racemase is provided in FIG. 7, as stated on page 4. The title of the application also is corrected in the substitute sequence listing, from "Mammalian Serine Protease" to "Mammalian Serine Racemase." This correction, too, is obvious, as the subject matter to which the application is directed is clearly mammalian serine racemase, not serine protease.

Substitute page 4, line 27 refers to SEQ ID NO:11 as the coding sequence of mouse serine racemase. This correction is obvious because there is only one nucleotide sequence shown in FIG. 7 and that particular nucleotide sequence is not otherwise depicted in the originally filed sequence listing.

Applicant respectfully requests that the International Searching Authority authorize rectification of the errors described above.

Respectfully submitted,

Dated:

4-21-00

By:

Lisa M. Hemmendinger

Lisa M. Hemmendinger  
Registration No. 42,653

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Washington, D.C. 20001-4597  
202-508-9100

chemiluminescence and HPLC assay. The experiment was replicated three times using different preparations with similar results.

**Figure 3.** Kinetic parameters of racemization reaction. Initial rate of racemase activity was measured at 37 °C in a medium containing 50 mM Tris-HCl (pH 8.0), 35 µg/ml purified enzyme, 1 mM EDTA, 2 mM DTT, 15 µM pyridoxal 5'-phosphate, and different concentrations of either L- or D- serine. The reaction was stopped after 2 hours, when less than 10% of the substrate was consumed. Values for  $K_m$  and  $V_{max}$  were calculated using the Michaelis-Menten equation. The values are representative of three experiments with different enzyme preparations.

**Figure 4.** Inhibition of serine racemase by PLP inhibitors and sulfhydryl oxidation. **Figure 4A.** Enzyme activity was monitored at 37 °C in a medium containing 50 mM Tris-HCl (pH 8.0), 20 mM L-serine, 40-100 µg/ml purified enzyme, 1 mM EDTA, 2 mM DTT, 10 µM pyridoxal 5'-phosphate and different concentrations of either aminooxyacetic acid (○) or hydroxylamine (●). **Figure 4B.** Reaction medium and conditions were as described in A, except that DTT was omitted from the last step of the enzyme preparation. The enzyme was preincubated for 10 minutes in the presence of different concentrations of oxidized glutathione (GSSG).

**Figure 5.** Absorption spectra of purified serine racemase. Purified enzyme (70 µg/ml) was preincubated for 10 minutes in medium containing 10 mM KPi (pH 7.2), 2 mM DTT, 1 mM EDTA, 10 µM PLP, either in the absence (Control) or in the presence of 1 mM aminooxyacetic acid (AOAA). The distinct peaks of absorbance at 420 and 340 nm were not observed in the presence of buffer alone or when bovine serum albumin was used instead of serine racemase.

**Figure 6.** Production of D-serine in culture medium of cells transfected with cDNA encoding full-length serine racemase (left) and in the transfected cells (right).

**Figure 7.** Complete coding sequence of mouse serine racemase (SEQ ID NO: 11) and its translated amino acid sequence (SEQ ID NO:8).

## **DETAILED DESCRIPTION OF THE INVENTION**

The inventors' isolation of serine racemase represents the first purification of an enzyme which converts L-serine to D-serine. Bacteria contain substantial levels of D-serine and many other D-amino acids. Though a number of amino acid

SEQUENCE LISTING

<110> Wolosker, Herman  
Takashashi, Maasaki  
Mothet, Jean-Pierre  
Ferris, Christopher  
Snyder, Solomon

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25.04.2000

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# PATENT COOPERATION TREATY

*ent'l BAK/LM4*  
*AMT*

From the INTERNATIONAL SEARCHING AUTHORITY

## PCT

To:  
BANNER & WITCOFF, LTD.  
Attn. KAGAN, Sarah A.  
1001 G Street, N.W.  
Eleventh Floor  
Washington, DC 20001-4597  
UNITED STATES OF AMERICA

**RECEIVED**

MAY 31 2000

**DOCKETED**

MAY 31 2000

*Article 19 Amend.  
due 7-23-00*

NOTIFICATION OF TRANSMITTAL OF  
THE INTERNATIONAL SEARCH REPORT  
OR THE DECLARATION

(PCT Rule 44.1)

Applicant's or agent's file reference <b>1107.85600</b>	Date of mailing (day/month/year) <b>23/05/2000</b>
International application No. <b>PCT/US 00/ 00938</b>	International filing date (day/month/year) <b>18/01/2000</b>
Applicant  <b>THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE</b>	

1. ☒ The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

**Filing of amendments and statement under Article 19:**

The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

**When?** The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

**Where?** Directly to the International Bureau of WIPO  
34, chemin des Colombettes  
1211 Geneva 20, Switzerland  
Facsimile No.: (41-22) 740.14.35

**For more detailed instructions,** see the notes on the accompanying sheet.

2. ☐ The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. ☐ **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.


4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau.

If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90bis.1 and 90bis.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority  European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer  <b>Mireille Claudepierre</b>
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These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

## INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

### What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

### When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

### Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been /is filed, see below.

### How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

### What documents must/may accompany the amendments?

#### Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether

- (i) the claim is unchanged;
- (ii) the claim is cancelled;
- (iii) the claim is new;
- (iv) the claim replaces one or more claims as filed;
- (v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:  
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."
2. [Where originally there were 15 claims and after amendment of all claims there are 11]:  
"Claims 1 to 15 replaced by amended claims 1 to 11."
3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:  
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or  
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."
4. [Where various kinds of amendments are made]:  
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

**"Statement under article 19(1)" (Rule 46.4)**

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

**It must be in the language in which the international application is to be published.**

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

**Consequence if a demand for international preliminary examination has already been filed**

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

**Consequence with regard to translation of the international application for entry into the national phase**

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

# PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference <b>1107.85600</b>	<b>FOR FURTHER ACTION</b> see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. <b>PCT/US 00/ 00938</b>	International filing date (day/month/year) <b>18/01/2000</b>	(Earliest) Priority Date (day/month/year) <b>19/01/1999</b>
Applicant  <b>THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE</b>		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

**1. Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing:

☒ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☒ furnished subsequently to this Authority in computer readable form.

☒ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☒ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☐ **Unity of Invention is lacking** (see Box II).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. \_\_\_\_\_

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☐ None of the figures.

## INTERNATIONAL SEARCH REPORT

International Application No.

US 00/00938

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/61 C12N9/90 C12N15/85 C12N5/10 C12Q1/533

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TAKUMA UO ET AL.: "Occurrence of pyridoxal 5'-phosphate-dependent Serine racemase in silkworm, Bombyx mori" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 246, no. 1, 8 May 1998 (1998-05-08), pages 31-34, XP002135965 ORLANDO, FL US abstract page 32, right-hand column, paragraph 2 -page 33, left-hand column, paragraph 3 page 34, left-hand column, last paragraph ---	1-13
X	GB 2 048 266 A (MITSUITOATSU CHEMICALS) 10 December 1980 (1980-12-10)  page 2, line 16 - line 50 --- -/--	1-6, 9-11, 24, 28, 32-38



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

## \* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&amp;" document member of the same patent family

Date of the actual completion of the international search

9 May 2000

Date of mailing of the international search report

23/05/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Montero Lopez, B

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DAVID S. DUNLOP ET AL.: "The origin and turnover of D-Serine in brain" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 235, no. 1, 9 June 1997 (1997-06-09), pages 26-30, XP002135966 ORLANDO, FL US abstract page 30, right-hand column, paragraph 2 - paragraph 3	1-13
P, X	--- HERMAN WOLOSKE ET AL.: "Serine racemase: a glial enzyme synthesizing D-Serine to regulate glutamate-N-methyl-D-aspartate neurotransmission" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 96, no. 23, 9 November 1999 (1999-11-09), pages 13409-13414, XP002135967 WASHINGTON US page 13410, left-hand column, paragraph 5 - page 13413, right-hand column, paragraph 2; figure 1	1-38
P, X	--- HERMAN WOLOSKE ET AL.: "Purification of Serine racemase: Bioynthesis of the neuromodulator D-Serine" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 96, no. 2, 19 January 1999 (1999-01-19), pages 721-725, XP002135968 WASHINGTON US page 722, right-hand column, paragraph 3 - page 725, left-hand column, last paragraph -----	1-13, 32-38

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

US 00/00938

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB 2048266 A	10-12-1980	JP 56134992 A	22-10-1981
		JP 1207308 C	11-05-1984
		JP 55148095 A	18-11-1980
		JP 57031438 B	05-07-1982
		JP 56005098 A	20-01-1981
		JP 61000070 B	06-01-1986
		AU 530435 B	14-07-1983
		AU 5797980 A	13-11-1980
		CA 1128443 A	27-07-1982
		CH 642947 A	15-05-1984
		DE 3017861 A	22-01-1981
		FR 2456140 A	05-12-1980
		IT 1145337 B	05-11-1986
		MX 6037 E	08-10-1984
		NL 8002603 A	11-11-1980
		US 4335209 A	15-06-1982

**PATENT COOPERATION TREATY**

*SAR/LMA*

From the RECEIVING OFFICE

<b>To:</b> SARAH A. KAGAN BANNER & WITCOFF, LTD. 1001 G STREET, N.W. ELEVENTH FLOOR WASHINGTON, DC 20001 4597 <b>BANNER &amp; WITCOFF LTD.</b>		<b>RECEIVED</b> <b>JUN 02 2000</b> <b>PCT</b> COMMUNICATION REGARDING EXTENSION OF TIME LIMIT (PCT Rule 26.2)
		Date of mailing (day/month/year) <b>01 JUN 2000</b>
Applicant's or agent's file reference <b>1107.85600</b>	<b>IMPORTANT COMMUNICATION</b>	
International application No. <b>PCT/US00/00938</b>	International filing date (day/month/year) <b>18 JAN 00</b>	
Applicant <b>THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE</b>		

1. In response to the applicant's request of 17 MAY 00, the time limit for replying to:

☐ the Invitation to Correct Defects (PCT/RO/106)

☐ (other) \_\_\_\_\_

has been extended as follows:

☐ extension of \_\_\_\_\_ months/days from \_\_\_\_\_

☒ extension until 19 MAY 00 (SUBMIT REGARDLESS)

2. ☐ No extension of the time limit is granted and the time limit remains as previously set.

*0110 7.85600*  
**Previously**  
**Docketed**  
*Correct Defect*  
*Filed 26 May 2000*

Name and mailing address of the receiving Office Assistant Commissioner for Patent Box PCT Washington, D.C. 20231 Attn:RO/US Facsimile No. 703-305-3230	Authorized officer <b>LARRY HAMMOND</b> <i>LH</i> Telephone No.
---	---

# PATENT COOPERATION TREATY

*AMH*

From the INTERNATIONAL SEARCHING AUTHORITY

## PCT

To:  
BANNER & WITCOFF, LTD.  
Attn. KAGAN, Sarah A.  
1001 G Street, N.W.  
Eleventh Floor  
Washington, DC 20001-4597  
UNITED STATES OF AMERICA

INVITATION TO FURNISH NUCLEOTIDE  
AND/OR AMINO ACID SEQUENCE LISTING  
COMPLYING WITH WIPO STANDARD ST25

(PCT Rule 13ter.1(a) and (c) and  
Administrative Instructions, Section 208 and Annex C)

Date of mailing (day/month/year)	03/04/2000
Applicant's or agent's file reference  1107.85600	<b>REPLY DUE</b>  within 1 months/ <del>days</del> from the above date of mailing
International application No.  PCT/US 00/00938	International filing date (day/month/year)  18/01/2000
Applicant  THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE	

1. The applicant is hereby **invited**, within the time limit indicated above, to furnish to this Authority:

- ☒ a nucleotide and/or amino acid sequence listing **in written form** complying with the standard provided for in Annex C of the Administrative Instructions, accompanied by a **statement** to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
- ☐ a **statement** to the effect that the sequence listing in written form, already furnished to this Authority, does not go beyond the disclosure in the international application as filed.
- ☒ a nucleotide and/or amino acid sequence listing **in computer readable form** complying with the standard provided for in Annex C of the Administrative Instructions, accompanied by a **statement** that the information recorded in computer readable form is identical to the written sequence listing.
- ☐ a **statement** that the information recorded in computer readable form (that computer readable form having already been furnished to this Authority) is identical to the written sequence listing.

2. **Failure to comply with this invitation** may result in this Authority not carrying out the international search to the extent that no meaningful search can be carried out.

3. Further observations (if necessary):

### IMPORTANT REMARK

The statements are legally required [See Suppl No 2 to Official Journal No 11/1998 (page 14, ¶ 37 & 40 and page 64 ¶ III.2)]

Name and mailing address of the International Searching Authority

 European Patent Office, P.B. 5818 Patentlaan 2  
NL-2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Zorka Bota-Madsen

*Z. Bota-Madsen*



Office Européen des Brevets  
Europäisches Patentamt  
European Patent Office

DG1  
DG1  
DG1

*Storage and Retrieval of Amino acid and Nucleotide Data*

Ms. Zorka Bota-Madsen  
P.B. 5818 Patentlaan 2  
Fax : + 31 70 340 39 92

NL-2280 HV Rijswijk  
Tel. : + 31 70 340 23 93

## **ANNEX**

Dear applicant/representative,

Present application contains amino acid/nucleotide sequences.

According to Supplement 2 to the Official Journal Nr.11/98 of the EPO [& Rule 5.2. PCT], if nucleotide / amino acid sequences are disclosed in a European/International patent application, the description shall contain a *sequence listing* complying with **WIPO standard ST. 25**.

**According to our verification the Sequence Listing on paper and on electronical medium which has been sent to us is not complete.**

**Namely, we observed that sequences given in the Sequence Listing do not correspond with the sequences given in the present application as originally filed. The SEQ.ID.NO. 9 does not correspond with Figure 7 as originally filed.**

**Please provide full DNA sequence of Figure 7 for SEQ.ID.NO.9.**

**The applicant is herewith invited to file the correct Sequence Listing, both on paper and in computer readable form.**

The expressions nucleotide and amino acid sequences mean an unbranched of **ten or more** contiguous nucleotides and an unbranched sequence of **four or more** contiguous amino acids. Nucleotide sequences shorter than 10 contiguous nucleotides and amino acids sequences shorter than 4 residues must not be included in a sequence listing.

**The ISA hereby invites the applicant to submit a sequence listing, with appropriate annotations for each sequence [where applicable], both on paper and in computer readable form, accompanied by the appropriate statements.**

Relating to this, we remind you that if these requirements are not met or not met in due time, the EPO does not perform the international search where a meaningful search cannot be carried out (Rule 13<sup>ter</sup>.1(c)PCT). In this case the international search report is replaced in full or in part by the statement under Article 17(2)(a)(ii)PCT.

*Moreover Rule 13<sup>ter</sup>(f) prescribes that a subsequently filed sequence listing, which is not a correction within the meaning of rule 26.4 PCT and which is not a rectification within the meaning of Rule 91.1.PCT of a sequence listing, shall not form part of the international application. In accordance herewith, the furnishing of a subsequently filed sequence listing does not give rise to an opportunity either to amend the description, claims and figures with a view to refer to said subsequently filed sequence listing or add it to the application as originally filed. The subsequently furnished listing will therefore normally not be forwarded to the international Bureau for publication purposes.*

We strongly recommend the applicant to use the **PatentIn** software to submit the sequence listing. (If problems arise with the download of the PatentIn software, a CD-ROM copy can be obtained through Ms. van Laar-Rabelink -Room 08-37, Tel +31 70 340 4440 ; Fax : +31 70 340 3992 ; E-mail : [epoline@epo.org](mailto:epoline@epo.org)), free of charge; Internet: <http://www.european-patent-office.org>.

The computer readable form of the Sequence Listing in ASCII format (text only) is mandatory. For further questions do not hesitate to contact us.

Please send sequence listing on paper and in computer-readable form preferably to the European Patent Office, Strand Program, Directorate Biotechnology (Dir 1212), Ms.Z.Bota-Madsen, Room S 02 N 28, Patentlaan 2, NL 2288 EE Rijswijk, The Netherland

**REMARK:**

The new PatentIn is available on our EPO website with following address:

**[www.european-patent-office.org/filingsoft/strand](http://www.european-patent-office.org/filingsoft/strand)**

Download is performed from that site .

Please read carefully the information provided on that site.

The downloaded install.exe file can be used  
for the installation of the new version from PatentIn.

W\_UPATIN.EXE is the file to start PatentIn

Would you encounter problems, please take contact  
with our Helpdesk [epoline@epo.org](mailto:epoline@epo.org).

## PCT COOPERATION TREA

PCT

From the INTERNATIONAL BUREAU

NOTIFICATION OF RECEIPT OF  
RECORD COPY

(PCT Rule 24.2(a))

RECEIVED

MAR 20 2000

BANNER &amp; WITCOFF LTD.

To:

KAGAN, Sarah, A.  
Banner & Witcoff, Ltd.  
11th floor  
1001 G Street, N.W.  
Washington, DC 20001-4597  
ÉTATS-UNIS D'AMÉRIQUE

Date of mailing (day/month/year)

06 March 2000 (06.03.00)

## IMPORTANT NOTIFICATION

Applicant's or agent's file reference

1107.85600

International application No.

PCT/US00/00938

The applicant is hereby notified that the International Bureau has received the record copy of the international application as detailed below.

Name(s) of the applicant(s) and State(s) for which they are applicants:

THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE (for all designated States  
except US)

SNYDER, Solomon, H. et al (for US)

International filing date : 18 January 2000 (18.01.00)

Priority date(s) claimed : 19 January 1999 (19.01.99)

21 July 1999 (21.07.99)

28 July 1999 (28.07.99)

Date of receipt of the record copy  
by the International Bureau

: 18 February 2000 (18.02.00)

List of designated Offices

:

AP : GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW

EA : AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

EP : AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

OA : BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

National : AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB,

GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK,

MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA,

ZW

The International Bureau of WIPO  
34, chemin des Colombettes  
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer:

R. Chrem

Telephone No. (41-22) 338.83.38

## Continuation of Form PCT/IB/301

## NOTIFICATION OF RECEIPT OF RECORD COPY

Date of mailing (day/month/year) 06 March 2000 (06.03.00)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference 1107.85600	International application No. PCT/US00/00938

**ATTENTION**

The applicant should carefully check the data appearing in this Notification. In case of any discrepancy between these data and the indications in the international application, the applicant should immediately inform the International Bureau.

In addition, the applicant's attention is drawn to the information contained in the Annex, relating to:

- ☒ time limits for entry into the national phase
- ☐ confirmation of precautionary designations
- ☒ requirements regarding priority documents

A copy of this Notification is being sent to the receiving Office and to the International Searching Authority.

## INFORMATION ON TIME LIMITS FOR ENTERING THE NATIONAL PHASE

The applicant is reminded that the "national phase" must be entered before each of the designated Offices indicated in the Notification of Receipt of Record Copy (Form PCT/IB/301) by paying national fees and furnishing translations, as prescribed by the applicable national laws.

The time limit for performing these procedural acts is **20 MONTHS** from the priority date or, for those designated States which the applicant elects in a demand for international preliminary examination or in a later election, **30 MONTHS** from the priority date, provided that the election is made before the expiration of 19 months from the priority date. Some designated (or elected) Offices have fixed time limits which expire even later than 20 or 30 months from the priority date. In other Offices an extension of time or grace period, in some cases upon payment of an additional fee, is available.

In addition to these procedural acts, the applicant may also have to comply with other special requirements applicable in certain Offices. **It is the applicant's responsibility** to ensure that the necessary steps to enter the national phase are taken in a timely fashion. Most designated Offices do not issue reminders to applicants in connection with the entry into the national phase.

**For detailed information about the procedural acts to be performed to enter the national phase before each designated Office, the applicable time limits and possible extensions of time or grace periods, and any other requirements, see the relevant Chapters of Volume II of the PCT Applicant's Guide. Information about the requirements for filing a demand for international preliminary examination is set out in Chapter IX of Volume I of the PCT Applicant's Guide.**

GR and ES became bound by PCT Chapter II on 7 September 1996 and 6 September 1997, respectively, and may, therefore, be elected in a demand or a later election filed on or after 7 September 1996 and 6 September 1997, respectively, regardless of the filing date of the international application. (See second paragraph above.)

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

## CONFIRMATION OF PRECAUTIONARY DESIGNATIONS

This notification lists only specific designations made under Rule 4.9(a) in the request. It is important to check that these designations are correct. Errors in designations can be corrected where precautionary designations have been made under Rule 4.9(b). The applicant is hereby reminded that any precautionary designations may be confirmed according to Rule 4.9(c) before the expiration of 15 months from the priority date. If it is not confirmed, it will automatically be regarded as withdrawn by the applicant. There will be no reminder and no invitation. Confirmation of a designation consists of the filing of a notice specifying the designated State concerned (with an indication of the kind of protection or treatment desired) and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.

## REQUIREMENTS REGARDING PRIORITY DOCUMENTS

For applicants who have not yet complied with the requirements regarding priority documents, the following is recalled.

Where the priority of an earlier national, regional or international application is claimed, the applicant must submit a copy of the said earlier application, certified by the authority with which it was filed ("the priority document") to the receiving Office (which will transmit it to the International Bureau) or directly to the International Bureau, before the expiration of 16 months from the priority date, provided that any such priority document may still be submitted to the International Bureau before that date of international publication of the international application, in which case that document will be considered to have been received by the International Bureau on the last day of the 16-month time limit (Rule 17.1(a)).

Where the priority document is issued by the receiving Office, the applicant may, instead of submitting the priority document, request the receiving Office to prepare and transmit the priority document to the International Bureau. Such request must be made before the expiration of the 16-month time limit and may be subjected by the receiving Office to the payment of a fee (Rule 17.1(b)).

If the priority document concerned is not submitted to the International Bureau or if the request to the receiving Office to prepare and transmit the priority document has not been made (and the corresponding fee, if any, paid) within the applicable time limit indicated under the preceding paragraphs, any designated State may disregard the priority claim, provided that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity to furnish the priority document within a time limit which is reasonable under the circumstances.

Where several priorities are claimed, the priority date to be considered for the purposes of computing the 16-month time limit is the filing date of the earliest application whose priority is claimed.

# PATENT COOPERATION TREATY

From the RECEIVING OFFICE

To:

SARAH A. KAGAN  
BANNER & WITCOFF, LTD.  
1001 G STREET, N.W.  
ELEVENTH FLOOR  
WASHINGTON DC 20001-4597

# PCT

**FEB 23 2000**

**NOTIFICATION CONCERNING PAYMENT  
OF PRESCRIBED FEES**

(PCT Rules 14, 15 and 16 and Administrative  
Instructions, Sections 304(a) and (b) and 323(b))

Date of mailing  
(day/month/year)

**17 FEB 2000**

Applicant's or agent's file reference  
1107.85600

**PAYMENT DUE**

See item 3 for time limits

International application No.

PCT/US00/00938

International filing date/Date of receipt  
(day/month/year)

18 JAN 00

Priority date (day/month/year)

19 JAN 99

Applicant

THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE

1. The applicant is hereby notified that this receiving Office has received:

☒ the payment of all the prescribed fees, and ☐ an overpayment, which will be refunded in due course.

☐ no or insufficient payment of the prescribed fees and the applicant is hereby invited to pay the balance due, as summarized under item 2, within the time limit(s) indicated under item 3.

2. Fees and payment calculation:

Total fees payable	Amount paid	= Balance
--------------------	-------------	-----------

☐ The details of the calculation are given in the Annex.

3. Time limit(s) for payment and amount(s) payable (Rules 14.1, 15.4 and 16.1(f)):

☐ within ONE MONTH from the date of receipt of the international application (for the transmittal fee (if any), the search fee, the basic fee and the designation fee). The amount payable for each fee is the amount applicable on the date of receipt of the international application.

☐ within ONE YEAR from the priority date (only for the designation fee and only if this time limit expires later than the above time limit).

---If the designation fee is paid within one month from the date of receipt of the international application, the amount payable is the amount applicable on that date of receipt.

---If the designation fee is paid within one year from the priority date but later than one month from the date of receipt of the international application, the amount payable is the amount applicable on the date of payment. The receiving Office should be consulted for the applicable amount.

☐ within 16 MONTHS from the priority date (only for the fee for priority document). The applicant's attention is drawn to the fact that the request made by the applicant under Rule 17.1(b) will be considered not to have been made unless the fee is paid within that time limit.

4. Additional observations (if necessary):

☐ The search copy will not be transmitted to the International Searching Authority until the search fee is paid (therefore the start of the international search will be delayed)(Rule 23.1(a) and (b)).

Name and mailing address of the receiving Office

Assistant Commissioner for Patents  
Box PCT  
Washington, D.C. 20231

Attn: RO/US

Facsimile No.

Authorized officer

**PERRY HACKLEY  
INTERNATIONAL DIVISION  
703-305-6517**

Telephone No.

**PERRY HACKLEY@USPTO.GOV**

# PATENT COOPERATION TREATY

From the RECEIVING OFFICE

To:

SARAH A. KAGAN  
BANNER & WITCOFF, LTD.  
1001 G STREET, N.W.  
ELEVENTH FLOOR  
WASHINGTON DC 20001-4597

## PCT

### NOTIFICATION OF THE INTERNATIONAL APPLICATION NUMBER AND OF THE INTERNATIONAL FILING DATE

(PCT Rule 20.5(c))

Date of mailing  
(day/month/year)

17 FEB 2000

Applicant's or agent's file reference  
1107.85600

#### IMPORTANT NOTIFICATION

International application No.

PCT/US00/00938

International filing date (day/month/year)

18 JAN 00

Priority date (day/month/year)

19 JAN 99

Applicant THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE

Title of the invention MAMMALIAN SERINE RACEMASE

1. The applicant is hereby notified that the international application has been accorded the international application number and the international filing date indicated above.

2. The applicant is further notified that the record copy of the international application:

17 FEB 2000



was transmitted to the International Bureau on \_\_\_\_\_



has not yet been transmitted to the International Bureau for the reason indicated below and a copy of this notification has been sent to the International Bureau\*\*



because the necessary national security clearance has not yet been obtained.



because (reason to be specified):

\* The International Bureau monitors the transmittal of the record copy by the receiving Office and will notify the applicant (with Form PCT/IB/301) of its receipt. Should the record copy not have been received by the expiration of 14 months from the priority date, the International Bureau will notify the applicant (Rule 22.1(c)).

#### 3. FOREIGN TRANSMITTAL LICENSE INFORMATION

Completed by: Perry Hackley



Additional license for foreign transmittal not required. This subject matter is covered by a license already granted on the equivalent U.S. national application. Refer to that license for information concerning its scope.



License for foreign transmittal not required. 37 CFR 5.11(e)(1) or 37 CFR 5.11(e)(2). However, a license may be required for additional subject matter. See 37 CFR 5.15(b).



Foreign transmittal license granted. 35 U.S.C. 184; 37 CFR 5.11 on 2-7-00 (date)



37 CFR 5.15(a)



37 CFR 5.15(b)

Name and mailing address of the receiving Office

Assistant Commissioner for Patents  
Box PCT  
Washington, D.C. 20231

Attn: RO/US

Facsimile No.

Authorized office

**PERRY HACKLEY**  
**INTERNATIONAL DIVISION**

**703-305-6517**

**PERRY HACKLEY@USPTO.GOV**

Telephone No.

# PATENT COOPERATION TREATY

*LH*

From the RECEIVING OFFICE

To:

SARAH A. KAGAN  
BANNER & WITCOFF, LTD.  
1001 G STREET, N.W.  
ELEVENTH FLOOR  
WASHINGTON DC 20001-4597

## PCT

### INVITATION TO CORRECT DEFECTS IN THE INTERNATIONAL APPLICATION

(PCT Articles 3(4)(i) and 14(1) and Rule 26)

Date of mailing  
(day/month/year)

**17 FEB 2000**

Applicant's or agent's file reference

1107.85600

**REPLY DUE**

within **ONE MONTH** from  
the above date of mailing

International application No.

PCT/US00/00938

International filing date  
(day/month/year)

18 JAN 00

Applicant

THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE

1. ☒ The applicant is hereby invited, within the time limit indicated above, to correct, in the international application as filed, the defects specified on the attached

☒ Annex A

☒ Annex B1 (text matter of the international application as filed)

☒ Annex C1 (drawings of the international application as filed)

2. ☐ The applicant is hereby invited, within the time limit indicated above, to correct, in the translation of the international application furnished under Rule 12.3, the defects specified on the attached

☐ Annex A

☐ Annex B2 (text matter of the translation of the international application)

☐ Annex C2 (drawings of the translation of the international application)

**DOCKETED**

FEB 25 2000

*FILE: 17/432000*

Additional observations (if necessary):

#### HOW TO CORRECT THE DEFECTS?

Correction must be submitted by filing a replacement sheet embodying the correction and a letter accompanying the replacement sheet, which shall draw attention to the difference between the replaced sheet and the replacement sheet. A correction may be stated in a letter only if it is of such a nature that it can be transferred from the letter to the record copy without adversely affecting the clarity and direct reproducibility of the sheet onto which the correction is to be transferred (Rule 26.4).

#### ATTENTION

Failure to correct the defects will result in the international application being considered withdrawn by this receiving Office (see Rule 26.5 for further details).

A copy of this invitation and any attachments has been sent to the International Bureau

☒ and the International Searching Authority.

Name and mailing address of the receiving Office

Assistant Commissioner for Patents  
Box PCT  
Washington, D.C. 20231

Attn: RO/US

Facsimile No.

Authorized officer

**PERRY HACKLEY**

**INTERNATIONAL DIVISION**

**703-305-6517**

Telephone No.

**PERRY.HACKLEY@USPTO.GOV**

18  
FEB  
2000

The receiving Office has found the following defects in the international application as filed:

1. As to **signature\*** of the international application (Rules 4.15 and 90.4), the request:
- a. ☐ is not signed.
  - b. ☐ is not signed by all applicants.
  - c. ☐ is not accompanied by the statement referred to in the check list in Box No. VIII of the request explaining the lack of the signature of an applicant for the designation of the United States of America.
  - d. ☒ is signed by what appears to be an agent/common representative but
    - ☒ the international application is not accompanied by a power of attorney appointing him.
    - ☐ the power of attorney accompanying the international application was not signed by all the applicants.
  - e. ☐ other (*specify*):

\* All applicants must sign, including inventors if they are also applicants (e.g. where the United States of America is designated).

2. As to indications concerning the **applicant**, the request (Rules 4.4 and 4.5):

- a. ☐ does not properly indicate the applicant's name (*specify*):
- b. ☐ does not indicate the applicant's address.
- c. ☐ does not properly indicate the applicant's address (*specify*):
- d. ☐ does not indicate the applicant's nationality.
- e. ☐ does not indicate the applicant's residence.
- f. ☐ other (*specify*):

3. As to the **language** of certain elements of the international application, other than the description and claims ( Rules 12.1(c) and 26.3ter(a) and (c)):

- a. ☐ the **request** is not in a language which is both a language accepted by this receiving Office and a language of publication, which is (are):
- b. ☐ the **text matter of the drawings** is not in the language in which the international application is to be published, which is:
- c. ☐ the **abstract** is not in the language in which the international application is to be published, which is:

4. The **title of the invention**:

- a. ☐ is not indicated in Box No. I of the request (Rule 4.1(a)).
- b. ☐ is not indicated at the top of the first sheet of the description (Rule 5.1(a)).
- c. ☐ as appearing in Box No. I of the request is not identical with the title heading the description (Rule 5.1(a)).

5. As to the **abstract** (Rule 8):

- ☐ the international application does not contain an abstract.

The receiving Office has found that, with regard to the presentation of the text matter of the international application as filed, the physical requirements are not complied with to the extent that compliance therewith is necessary for:

1. ☒ reasonably uniform international publication (Rules 11 and 26.3(a)(i)) (defects to be specified):

	Request	Description	Claims	Abstract
a. <input type="checkbox"/> The sheets do not admit of direct reproduction.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
b. <input type="checkbox"/> The element does not commence on a new sheet.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
c. <input type="checkbox"/> Sheets are not free from creases, cracks, folds.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
d. <input type="checkbox"/> Sheets are not used in the upright position.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
e. <input type="checkbox"/> One side of the sheets is not left unused.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
f. <input type="checkbox"/> The paper of the sheets is not flexible/strong/white/smooth/non-shiny/durable.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
g. <input type="checkbox"/> The sheets are not connected as prescribed (Rule 11.4(b)).	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
h. <input type="checkbox"/> Sheets are not A4 size (29.7cm x 21cm).	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
i. <input checked="" type="checkbox"/> The minimum margins on the sheets are not as prescribed (top: 2cm; left side: 2.5cm; right side: 2cm; bottom: 2cm). <i>p. 28</i>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
j. <input type="checkbox"/> The file reference number indicated on the sheets does not appear in the left-hand corner of the sheets, within 1.5cm of the top of the sheets.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
k. <input type="checkbox"/> The file reference number exceeds the maximum of 12 characters.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
l. <input type="checkbox"/> The sheets of the description, claims and abstract are not numbered in consecutive Arabic numerals.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
m. <input type="checkbox"/> The sheet numbers are not centered at the top or bottom of the sheets.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
n. <input type="checkbox"/> The sheet numbers are in the margin (see i. above for the size of the margins).	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
o. <input type="checkbox"/> The text matter is not typed or printed.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
p. <input type="checkbox"/> The typing on the sheets is not 1.5-spaced.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
q. <input type="checkbox"/> The characters in the text matter on the sheets are less than 0.21 cm high in capital letters.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
r. <input type="checkbox"/> The text matter on the sheets is not in dark, indelible color.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
s. <input type="checkbox"/> The element contains drawings.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
t. <input type="checkbox"/> The sheets contain alterations/overwritings/interlineations/too many erasures.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
u. <input checked="" type="checkbox"/> The sheets contain photocopy marks. <i>p. 28, 29</i>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

2. ☐ satisfactory reproduction (Rules 11 and 26.3(b)(i)).

Further observation (if necessary):

*SKewed PAGE  
p. 28*

*\* NEW SHEET  
REQUIRED*

The receiving Office has found that, with regard to the presentation of the drawings of the international application as filed, the physical requirements are not complied with to the extent that compliance therewith is necessary for:

1. ☒ reasonably uniform international publication (Rules 11 and 26.3(a)(i)) (defects to be specified):

**Sheets containing drawings:**

- a. ☐ the sheets do not admit of direct reproduction.
- b. ☐ the sheets are not free from creases, cracks, folds.
- c. ☐ one side of the sheets is not left unused.
- d. ☐ the paper of the sheets is not flexible/strong/white/smooth/non-shiny/durable.
- e. ☐ the drawings do not commence on a new sheet.
- f. ☐ the sheets are not connected as prescribed (Rule 11.4(b)).
- g. ☐ the sheets are not A4 size (29.7cm x 21cm).
- h. ☒ the minimum margins on the sheets are not as prescribed (top: 2.5cm; left side: 2.5cm; right side: 1.5cm; bottom: 1cm). *Fig 2-4*
- i. ☐ the file reference number indicated on the sheets does not appear in the left-hand corner of the sheets, within 1.5cm of the top of the sheets.
- j. ☐ the file reference number exceeds the maximum of 12 characters.
- k. ☐ the sheets are not free from frames around usable or used surfaces.
- l. ☒ the sheets are not numbered in consecutive Arabic numerals (e.g. 1/3, 2/3, 3/3). *ALL*
- m. ☐ the sheet numbers are not centered at the top or bottom of the sheets.
- n. ☐ the sheet numbers are in the margin (see h. above for the size of the margins).
- o. ☐ the sheets contain alterations/overwritings/interlineations/too many erasures.
- p. ☒ the sheets contain photocopy marks. *ALL*

**Drawings (Rule 11.13):**

- a. ☐ do not admit of direct reproduction.
- b. ☐ contain unnecessary text matter.
- c. ☐ contain words so placed as to prevent translation without interference with lines thereof.
- d. ☐ are not executed in durable black color; the lines are not uniformly thick and well-defined.
- e. ☐ contain cross-sections not properly hatched.
- f. ☐ would not be properly distinguishable in reduced reproduction.
- g. ☐ contain scales not represented graphically.
- h. ☐ contain numbers, letters and reference lines lacking simplicity and clarity.
- i. ☐ contain lines drafted without the aid of drafting instruments.
- j. ☐ contain disproportionate elements of a figure not necessary for clarity.
- k. ☐ contain numbers and letters of height less than 0.32 cm.
- l. ☐ contain letters not conforming to the Latin, and where customary, Greek alphabets.
- m. ☐ contain figures on two or more sheets which form a single complete figure but which are not able to be assembled without concealing parts thereof.
- n. ☐ contain figures which are not properly arranged and clearly separated.
- o. ☐ contain different figures not numbered in consecutive Arabic numerals.
- p. ☐ contain different figures not numbered independent of the numbering of the sheets.
- q. ☐ are not restricted to reference signs mentioned in the description.
- r. ☐ do not contain reference signs that are mentioned in the description.
- s. ☐ contain the same feature denoted by different reference signs.
- t. ☐ are not arranged in an upright position, clearly separated from one another.
- u. ☐ are not presented sideways with the top of the figures at the left side of the sheets.

2. ☐ satisfactory reproduction (Rules 11 and 26.3(b)(i)).

Further observations (if necessary):

*\* NEW DRAWINGS  
REQUIRED*

# PATENT COOPERATION TREATY

From the RECEIVING OFFICE

To:

SARAH A. KAGAN  
BANNER & WITCOFF, LTD.  
1001 G STREET, N.W.  
ELEVENTH FLOOR  
WASHINGTON DC 20001-4597

## PCT

NOTIFICATION REGARDING CERTAIN  
CORRECTIONS MADE *EX OFFICIO*

(PCT Administrative Instructions, Section 327)

Date of mailing  
(day/month/year)

17 FEB 2000

Applicant's or agent's file reference  
1107.85600

**REPLY DUE**

NONE

However, see paragraph 3 below

International application No.  
PCT/US00/00938

International filing date  
(day/month/year)

18 JAN 00

Applicant

THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE

1. The applicant is hereby notified that this receiving Office has corrected formal defects in the international application *ex officio*, as shown on the attached copy of:



the request, sheet No.:

6



the description, sheet No.:



the claims, sheet No.:



the drawings, sheet No.:



other (*specify*):

2. If the applicant agrees with these corrections, no further action is required in this regard.

3. In case of disagreement with these corrections, the applicant should promptly inform this receiving Office accordingly.

Name and mailing address of the receiving Office

Assistant Commissioner for Patents  
Box PCT  
Washington, D.C. 20231

Attn: RO/US

Facsimile No.

Authorized officer

PERRY HACKLEY

INTERNATIONAL DIVISION

703-305-6517

PERRY.HACKLEY@USPTO.GOV

Telephone No.

TO

SARAH A. KAGAN  
BANNER & WITCOFF, LTD.  
1001 G STREET, N.W.  
ELEVENTH FLOOR  
WASHINGTON DC 20001-4597

UNITED STATES DESIGNATED/ELECTED  
OFFICE (DO/EO/US)

NOTIFICATION OF STATUS OF  
REQUIREMENTS UNDER 35 U.S.C.371

DATE OF MAILING

17 FEB 2000

FILE REFERENCE

1107.85600

## IDENTIFICATION OF INTERNATIONAL APPLICATION

International Application Number

PCT/US00/00938

International Filing Date

18 JAN 00

Priority Date Claimed

19 JAN 99

Applicant for DO/EO/US

SNYDER, SOLOMON H.

## NOTIFICATION

The applicant is hereby advised that the U.S. Patent and Trademark Office in its capacity as ☒ Designated Office ☐ Elected Office has received the following items as of the date of mailing indicated above.

1. ☐ U.S. National fee [35 U.S.C.371 (c) (1)]
2. ☐ Oath of declaration [35 U.S.C.371 (c) (4)]
3. ☒ Copy of International application as filed [35 U.S.C.371 (c) (2)]
4. ☐ Translation of Application [35 U.S.C.371 (c) (2)]
5. ☐ Amendments under PCT Article 19 [35 U.S.C.371 (c) (3)]
6. ☐ Translation of PCT Article 19 Amendments [35 U.S.C.371 (c) (3)]
7. ☐ Search Report or Declaration under PCT Article 17(2) [35 U.S.C.371 (a)]
8. ☐ International Preliminary Examination Report and its Annexes, if any, under PCT Article 36(3) (a) [35 U.S.C.371 (a)]
9. ☐ Translation of Annexes to the International Preliminary Examination Report under PCT Article 36(3) (b) [35 U.S.C.371 (c) (5)]
10. ☐ Other items received:
  - ☐ Assignment Document ☐ Prior Art Statement ☐ Preliminary Amendment
- A. ☐ Requirements for U.S. National processing have been met. Processing will commence
  - ☐ at the expiration of the applicable time limit under either
    - ☐ PCT Article 22 [35 U.S.C.371 (b)] or
    - ☐ PCT Article 39 [35 U.S.C.371 (b)]
  - ☐ on the date indicated below under the provisions of 35 U.S.C.371 (f)

U.S. NATIONAL SERIAL#

DATE UNDER 35 U.S.C.102(e)

DATE OF COMMENCEMENT OF  
NATIONAL PROCESSING

*All correspondence submitted after the date of commencement of U.S. National processing indicated above should refer to the U.S. National Serial Number and the appropriate U.S. National processing organization or Officer.*

- B. ☐ As the above identified application has been accepted for U.S. National processing under the provisions of 35 U.S.C.371 (f) before expiration of the applicable time limit under ☐ PCT Article 22 ☐ PCT Article 39, applicant is reminded that
- ☐ Amendments under PCT Article 19 and/or
  - ☐ the International Preliminary Examination Report and its Annexes, if any, under PCT Article 36(3) (a), and (b)
- and any translation thereof, if applicable, must be submitted to the Patent and Trademark Office as soon as they are available.

International application N . \*

PCT/US 00/00908

International filing date

18 JAN 2000

Priority Date Claimed

19 JAN 1999

- C. ☒ In order that U.S. National processing may begin, certain items must be received by the DO/EO/US by the expiration of applicable time limit under
- ☒ PCT Article 22 or
- ☒ PCT Article 39.
- Specifically:
- ☒ 1. U.S. National Fee
- ☒ 2. Oath or Declaration
- ☐ 3. Copy of Application
- ☐ 4. Translation of application
- ☒ 5. Amendments under PCT Article 19, if any
- ☐ 6. Translation of PCT Article 19 Amendments, if applicable
- ☐ 7. Search Report or PCT Article 17(2) declaration
- ☐ 8. International Preliminary Examination Report and its Annexes, if any, under PCT Article 36(3)(a), if applicable
- ☐ 9. Translation of Annexes to the International Preliminary Examination Report under PCT Article 36(3)(b), if applicable

**THE ABOVE CHECK ITEMS MUST BE TIMELY RECEIVED TO AVOID ABANDONMENT OF THE APPLICATION.**  
[35. U.S.C. 371(d)]

D. Further information for the applicant:

This is only a reminder.

## UNITED STATES DESIGNATED/ELECTED OFFICE

Address Only:

Assistant Commissioner for Patent

Box PCT

Washington, D.C. 20231 Attn: RO/US

Authorized Officer

PERRY HACKLEY

INTERNATIONAL DIVISION

703-305-6517

PERRY.HACKLEY@USPTO.GOV

<b>Box No. VI PRIORITY CLAIM</b>		<input type="checkbox"/> Further priority claims are indicated in the Supplemental Box.		
		Where earlier application is:		
Filing date of earlier application (day/month/year)	Number of earlier application	national application: country	regional application: * regional Office	international application: receiving Office
item (1) 28 July 1999 (28.07.99)	60/145,953	US		
item (2) 21 July 1999 (21.07.99)	60/144,839	US		
item (3) 19 January 1999 (19.01.99)	60/116,333	US		

[X] The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) (only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office) identified above as item(s): (1), (2) and (3)

\* Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.

**Box No. VII INTERNATIONAL SEARCHING AUTHORITY**

Choice of International Searching Authority (ISA) (if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):  
ISA/EP

Request to use results of earlier search; reference to that search (if an earlier search has been carried out by or requested from the International Searching Authority):  
Date (day/month/year) Number Country (or regional Office)

**Box No. VIII CHECK LIST; LANGUAGE OF FILING**

This international application contains the following number of sheets:

request : 6 sheets  
 description (excluding sequence listing part) : 31 sheets  
 claims : 3 sheets  
 abstract : 1 sheet  
 drawings : 7 sheets  
 sequence listing part of description : 7 sheets  
 Total number of sheets : 55 sheets

This international application is accompanied by the item(s) marked below:

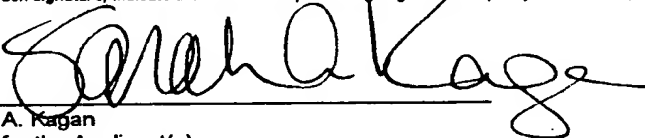
- ☒ fee calculation sheet (duplicate)
- ☐ separate signed power of attorney
- ☐ copy of general power of attorney; reference number, if any:
- ☐ statement explaining lack of signature
- ☐ priority document(s) identified in Box No. VI as item(s):
- ☐ translation of international application into (language):
- ☐ separate indications concerning deposited microorganism or other biological material
- ☐ nucleotide and/or amino acid sequence listing in computer readable form
- ☒ other (specify): Transmittal

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: ENGLISH

**Box No. IX SIGNATURE OF APPLICANT OR AGENT**

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

  
 Sarah A. Kagan  
 Agent for the Applicant(s)

For receiving Office use only

1. Date of actual receipt of the international application: <b>424 Rec'd PCT/PTO 18 JAN 2000</b> (18.01.00)	2. Drawings: <input type="checkbox"/> received: <input type="checkbox"/> not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application: -	
4. Date of timely receipt of the required corrections under PCT Article 11(2):	
5. International Searching Authority (if two or more are competent): ISA/EP	6. <input type="checkbox"/> Transmittal of search copy delayed until search fee is paid

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

# PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

**PCT REVIEWED**

To:  
BANNER & WITCOFF, LTD.  
Attn. KAGAN, Sarah A.  
1001 G Street, N.W.  
Eleventh Floor  
Washington, DC 20001-4597  
UNITED STATES OF AMERICA

**RECEIVED** NOTIFICATION OF RECEIPT  
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19/01/1999

Applicant

THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE

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(54) Title: MAMMALIAN SERINE RACEMASE			
(57) Abstract			
<p>High levels of D-serine occur in mammalian brain, where it appears to be an endogenous ligand of the "glycine site" of NMDA receptors. We have purified from rat brain a soluble enzyme that catalyzes the direct racemization of L-serine to D-serine. Purified serine racemase has a molecular weight of 37 kDa and requires pyridoxal 5'-phosphate for its activity. The enzyme is highly selective toward L-serine, failing to racemize any other amino acid tested. We have also identified polynucleotide sequences which encode mammalian, including human, serine racemase. Compounds which modulate the activity of mammalian serine racemase are useful for treating conditions and diseases which involve overstimulation of NMDA receptors, such as stroke and various neurodegenerative diseases.</p>			

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## MAMMALIAN SERINE RACEMASE

The U.S. Government has a paid-up license in this invention and the right in limited circumstances to require the patent owner to license others on reasonable terms as provided for by the terms of USPHS grant MH-18501 and Research Scientist Award DA00074 awarded by the National Institutes of Health.

### TECHNICAL AREA OF THE INVENTION

This invention relates to the area of mammalian amino acid racemase enzymes.

### BACKGROUND OF THE INVENTION

D-amino acids are prominent in bacteria, and there have been occasional reports of D-amino acids in invertebrates (1, 2), whereas animal tissues were believed to contain L-amino acids exclusively. Recently, however, D-serine (3-6) and D-aspartate (7, 8) were reported in mammalian tissues, especially in the nervous system. Utilizing highly selective antibodies, we localized D-aspartate to neuroendocrine tissues (9), while the immunohistochemical localizations of D-serine closely resemble N-methyl-D-aspartate (NMDA) receptors for the neurotransmitter glutamate, consistent with chemical measurements of the distribution of D-serine (10, 11).

Glutamate cannot activate the NMDA receptor in the absence of added glycine, which indicates a "glycine site" for the receptor (12, 13). D-Serine is up to three times more potent than glycine at this site (14), suggesting that D-serine is the endogenous ligand for this site. D-Serine is localized exclusively to Type II astrocytes, a form of glia concentrated in gray matter in the same areas of the brain as

NMDA receptors (10). Stimulation of the kainate subtype of glutamate receptors releases D-serine from Type II astrocytes, which implies that synaptic release of glutamate triggers release of D-serine from the astrocytes to activate NMDA receptors physiologically (10). While in most parts of the brain the distribution of D-serine resembles NMDA receptors far better than does the distribution of glycine, in some areas glycine and NMDA receptors are co-localized, suggesting that D-serine is the predominant ligand for the receptor in most brain areas but that glycine serves this purpose in some sites (11).

Activation of NMDA receptors is an important pathologic event in stroke and several neurodegenerative diseases, leading to cell death. Decreased activation of NMDA receptors can thus have a beneficial effect in the treatment of any condition or disease that includes acute or chronic neuronal death or dysfunction mediated by overactivation of NMDA receptors. Overactivation of NMDA receptors is involved in stroke, epilepsy, and chronic neurodegenerative diseases such as Parkinson's disease, Huntington's disease, motor neuron diseases, and Alzheimer's disease. Thus, there is a need in the art to determine how D-serine is formed in the brain, so that its concentration in NMDA-related diseases can be regulated.

#### **SUMMARY OF THE INVENTION**

It is an object of this invention to provide isolated mammalian serine racemase protein and polynucleotide molecules, as well as methods of producing these molecules. These and other objects of the invention are provided by one or more of the embodiments described below.

One embodiment of the invention is a preparation of an isolated mammalian serine racemase having a specific activity of at least 0.003  $\mu$ mole L-serine/mg/hour.

Another embodiment of the invention is an isolated and purified polynucleotide molecule which encodes a mammalian serine racemase.

Still another embodiment of the invention is a host cell comprising an expression construct which comprises a polynucleotide molecule encoding a murine serine racemase.

Even another embodiment of the invention is a method of producing a mammalian serine racemase. A host cell comprising an expression construct which

comprises a polynucleotide molecule encoding a mammalian serine racemase is cultured in a culture medium. Mammalian serine racemase is recovered from the culture medium or the host cell.

5 Yet another embodiment of the invention is a method to screen compounds to identify candidate therapeutic agents. A test compound is contacted with a mammalian serine racemase. Activity of the mammalian serine racemase is assayed. A test compound is identified as a candidate therapeutic agent if it modulates the activity of the mammalian serine racemase.

10 The invention thus provides mammalian serine racemase molecules, polynucleotide sequences encoding the molecules, host cells, methods of producing mammalian serine racemase, and methods of screening test compounds to identify modulators of mammalian serine racemase. Modulators of mammalian serine racemase molecules can be used therapeutically, *inter alia*, to treat acute or chronic neural death or dysfunction mediated by overactivation of NMDA receptors.

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#### **BRIEF DESCRIPTION OF THE DRAWINGS**

**Figure 1.** SDS/PAGE analysis of purified serine racemase. A 12% polyacrylamide gel was stained with Coomassie blue. Lane 1, molecular weight markers: myosin (200 kDa);  $\beta$ -galactosidase (116.7 kDa); phosphorylase b (97.4 kDa); bovine serum albumin (66.3 kDa); glutamic dehydrogenase (55.4 kDa); lactate dehydrogenase (36.5 kDa); carbonic anhydrase (31 kDa); trypsin inhibitor (21.5 kDa). Lane 2, mono Q column eluate containing 1  $\mu$ g protein. Lane 3, Hydroxyapatite column eluate containing 0.5  $\mu$ g of purified protein. Silver staining of the purified preparation showed no additional bands.

25 **Figure 2.** pH and temperature-dependence of racemase activity. **Figure 2A.** Racemase activity was assayed at 37 °C in media containing 50 mM MES-Tris (pH 6.0 to 6.5), 50 mM Tris-HCl (pH 6.8 to 8.8) or 50 mM CAPS-NaOH (pH 9 to 10.5), 20 mM L-serine, 40-100  $\mu$ g/ml purified enzyme, 1 mM EDTA, 2 mM DTT, and 15  $\mu$ M pyridoxal 5'-phosphate. **Figure 2B.** Racemase activity was assayed at different temperatures in a medium containing 50 mM Tris-HCl (pH 8.0), 20 mM L-serine, 40-100  $\mu$ g/ml purified enzyme, 1 mM EDTA, 2 mM DTT, and 15  $\mu$ M pyridoxal 5'-phosphate. The reaction was stopped after 4 hours and analyzed by both

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chemiluminescence and HPLC assay. The experiment was replicated three times using different preparations with similar results.

**Figure 3.** Kinetic parameters of racemization reaction. Initial rate of racemase activity was measured at 37 °C in a medium containing 50 mM Tris-HCl (pH 8.0), 35 µg/ml purified enzyme, 1 mM EDTA, 2 mM DTT, 15 µM pyridoxal 5'-phosphate, and different concentrations of either L- or D- serine. The reaction was stopped after 2 hours, when less than 10% of the substrate was consumed. Values for  $K_m$  and  $V_{max}$  were calculated using the Michaelis-Menten equation. The values are representative of three experiments with different enzyme preparations.

**Figure 4.** Inhibition of serine racemase by PLP inhibitors and sulfhydryl oxidation. **Figure 4A.** Enzyme activity was monitored at 37 °C in a medium containing 50 mM Tris-HCl (pH 8.0), 20 mM L-serine, 40-100 µg/ml purified enzyme, 1 mM EDTA, 2 mM DTT, 10 µM pyridoxal 5'-phosphate and different concentrations of either aminooxyacetic acid (○) or hydroxylamine (●). **Figure 4B.** Reaction medium and conditions were as described in A, except that DTT was omitted from the last step of the enzyme preparation. The enzyme was preincubated for 10 minutes in the presence of different concentrations of oxidized glutathione (GSSG).

**Figure 5.** Absorption spectra of purified serine racemase. Purified enzyme (70 µg/ml) was preincubated for 10 minutes in medium containing 10 mM KPi (pH 7.2), 2 mM DTT, 1 mM EDTA, 10 µM PLP, either in the absence (Control) or in the presence of 1 mM aminooxyacetic acid (AOAA). The distinct peaks of absorbance at 420 and 340 nm were not observed in the presence of buffer alone or when bovine serum albumin was used instead of serine racemase.

**Figure 6.** Production of D-serine in culture medium of cells transfected with cDNA encoding full-length serine racemase (left) and in the transfected cells (right).

**Figure 7.** Complete coding sequence of mouse serine racemase (SEQ ID NO: 9) and its translated amino acid sequence.

## **DETAILED DESCRIPTION OF THE INVENTION**

The inventors' isolation of serine racemase represents the first purification of an enzyme which converts L-serine to D-serine. Bacteria contain substantial levels of D-serine and many other D-amino acids. Though a number of amino acid

racemases have been purified for bacteria, no serine-specific enzyme has been previously identified (19-21).

Serine racemase appears to be a very conserved enzyme. For example, the pH optimum in the rat brain enzyme we have purified, as well as its requirement for PLP and behavior in chromatographic systems, resemble the activity characterized in crude preparations of silkworm (18). Bacterial amino acid racemases display properties resembling serine racemase including  $K_m$  values, alkaline pH optimum, and the requirement for PLP. The alkaline pH optimum, might reflect the mechanism of racemization, as PLP non-enzymatically racemizes amino acids at alkaline pH (22).

Serine racemase displays a  $K_m$  value in the direction L- to D-serine which resembles brain levels of L-serine and which favors the physiologic synthesis of D-serine. Because of the much higher  $K_m$  value in the direction D- to L-serine, under physiological conditions the enzyme should predominantly make D-serine.

Mammalian serine racemase can be isolated from homogenates of mammalian brain, such as rat, mouse, or preferably human brain. Other forms of mammalian serine racemase can be isolated from brain homogenates of other mammals, such as monkey, pig, cow, sheep, goat, guinea pig, and the like. The enzyme can be purified, partially or to homogeneity, using all or part of the method described in Example 1. This method employs, sequentially, ammonium sulfate fractionation, butyl-sepharose, Q-sepharose, mon-Q, and hydroxyapatite chromatography steps (Table 1).

Preferably, a preparation of isolated mammalian serine racemase is able to convert L-serine to D-serine with a specific activity of at least 0.003, .025, 0.075, 1, 2.5, or 5  $\mu$ mole of L-serine/mg/hr. The specific activity of isolated mammalian serine racemase can be determined *inter alia* by the assay described in Example 2. Other methods as are known in the art can also be employed.

Serine racemase is a relatively small soluble protein of 37 kDa. Murine serine racemase has the amino acid sequence shown in SEQ ID NO: 8. Rat serine racemase comprises the amino acid sequences shown in SEQ ID NOS: 6 and 7. Human serine racemase comprises amino acid sequences encoded by the nucleotide sequences shown in SEQ ID NO: 2, 3, or 9. The protein contains a pyridoxal 5' phosphate binding region (ELFQKTGSFKIRGA, amino acids 47-60 of SEQ ID NO: 8), which supports the biochemical prediction that the serine racemase is a pyridoxal phosphate

binding protein (Figures 4 and 5).

The invention also provides polypeptide fragments of mammalian serine racemase. Polypeptide fragments may contain less than full-length mammalian serine racemase and can contain at least 6, 8, 10, 13, 25, 27, 50, 100, 121, 150, 200, 250, or 300 contiguous amino acids selected from SEQ ID NOS: 6, 7, 8, or 10 or amino acid sequences encoded by the nucleotide sequences shown in SEQ ID NO: 1, 2, 3, or 9. One such polypeptide fragment is the pyridoxal 5' phosphate binding region (amino acids 47-60 of SEQ ID NO: 8). Other polypeptide fragments of interest can be identified using routine protein analysis techniques known in the art. These techniques include, but are not limited to, hydrophobicity and hydrophilicity plots, homology searches for various motifs, antigenic indices, and standard algorithms such as those disclosed in Harlow & Lane, ANTIBODIES- A LABORATORY MANUAL (Cold Spring Harbor Laboratory, 1988). Enzymes can be used to generate mammalian serine racemase polypeptides by enzymatic proteolysis of full-length mammalian serine racemase. Polypeptide fragments can be used to generate antibodies, which in turn can be used to localize the racemase in tissues.

Mammalian serine racemase protein and polypeptides can also be produced by recombinant DNA methods or by synthetic chemical methods. For production of recombinant serine racemase, coding sequences such as those selected from the nucleotide sequences shown in SEQ ID NOS: 1, 2, 3, or 9 can be expressed in known prokaryotic or eukaryotic expression systems. Bacterial, yeast, insect, or mammalian expression systems can be used, as is known in the art.

Alternatively, synthetic chemical methods, such as solid phase peptide synthesis, can be used to synthesize mammalian serine racemase protein or polypeptides. General means for the production of peptides, analogs or derivatives are outlined in B. Weinstein, ed., CHEMISTRY AND BIOCHEMISTRY OF AMINO ACIDS, PEPTIDES, AND PROTEINS -- A SURVEY OF RECENT DEVELOPMENTS (1983).

Mammalian serine racemase proteins, however produced, can contain alterations in amino acid sequence relative to the amino acid sequences encoded by SEQ ID NOS: 1, 2, 3, or 9 which do not affect the serine racemase activity of the protein. Guidance in determining which amino acid residues may be conservatively substituted, inserted, or deleted without abolishing serine racemase activity can be

found using computer programs well known in the art, such as DNASTAR software. Whether an amino acid change results in a functional serine racemase can readily be determined by assaying serine racemase activity as described in Example 2.

Preferred mammalian serine racemase proteins have amino acid sequences which are at least 85%, 90%, 95%, 96%, or 97% identical to the amino acid sequences encoded by a polynucleotide having a coding sequence as shown in SEQ ID NOS: 1, 2, 3, or 9. More preferably, the molecules are at least 98% or 99% identical. Percent identity is determined according to the Smith-Waterman homology search algorithm, using an affine gap search with the following parameters: a gap open penalty of 12 and a gap extension penalty of 1. The Smith-Waterman homology search algorithm is taught in Smith and Waterman, *Adv. Appl. Math.* (1981) 2: 482-489.

Fusion proteins comprising at least 6, 8, 10, 13, 25, 27, 50, 100, 121, 150, 200, 250, or 300 contiguous amino acids selected from SEQ ID NOS: 6, 7, 8, or 10 or amino acid sequences encoded by SEQ ID NO: 1, 2, 3, or 9 can also be constructed. Fusion proteins are useful for generating antibodies against mammalian serine racemase amino acid sequences and for use in various assay systems. For example, mammalian serine racemase fusion proteins can be used to identify proteins which interact with the enzyme and which influence its racemase activity. Physical methods, such as protein affinity chromatography, or library-based assays for protein-protein interactions, such as the yeast two-hybrid or phage display systems, can also be used for this purpose. Such methods are well known in the art and can also be used to screen drugs.

A mammalian serine racemase fusion protein comprises two protein segments fused together by means of a peptide bond. The first protein segment consists of at least 6, 8, 10, 13, 25, 27, 50, 100, 121, 150, 200, 250, or 300 contiguous amino acids selected from SEQ ID NOS: 6, 7, 8, or 10 or amino acid sequences encoded by SEQ ID NO: 1, 2, 3, or 9. The first protein segment can also be a full-length mammalian serine racemase protein. The first protein segment can be N-terminal or C-terminal, as is convenient.

The second protein segment can be a full-length protein or a protein fragment or polypeptide. Proteins commonly used in fusion protein construction include  $\beta$ -galactosidase,  $\beta$ -glucuronidase, green fluorescent protein (GFP), autofluorescent

proteins, including blue fluorescent protein (BFP), glutathione-S-transferase (GST), luciferase, horseradish peroxidase (HRP), and chloramphenicol acetyltransferase (CAT). Epitope tags can be used in fusion protein constructions, including histidine (His) tags, FLAG tags (Kodak), influenza hemagglutinin (HA) tags, Myc tags, VSV-G tags, and thioredoxin (Trx) tags. Other fusion constructions can include maltose binding protein (MBP), S-tag, Lex A DNA binding domain (DBD) fusions, GAL4 DNA binding domain fusions, and herpes simplex virus (HSV) BP16 protein fusions.

Mammalian serine racemase fusion proteins can be made by covalently linking the first and second protein segments or by standard procedures in the art of recombinant DNA technology. Recombinant DNA methods can be used to prepare fusion proteins, for example, by making a DNA construct which comprises coding sequences selected from SEQ ID NOS: 1, 2, 3, or 9 in proper reading frame with nucleotides encoding the second protein segment and expressing the DNA construct in a host cell, as is known in the art. Many kits for constructing fusion proteins are commercially available from companies such as Promega Corporation (Madison, WI), Stratagene (La Jolla, CA), Clontech (Mountain View, CA), Santa Cruz Biotechnology (Santa Cruz, CA), MBL International Corporation (MIC; Watertown, MA), and Quantum Biotechnologies (Montreal, Canada; 1-888-DNA-KITS).

Isolated mammalian serine racemase proteins, polypeptides, or fusion proteins can be used as immunogens, to obtain a preparation of antibodies which specifically bind to epitopes of mammalian serine racemase. The antibodies can be used, *inter alia*, to detect mammalian serine racemase in mammalian brain tissue or in fractions thereof. The antibodies can also be used to detect the presence of mutations in a gene encoding a mammalian serine racemase which result in under- or over-expression of the enzyme or in expression of an enzyme with altered size or electrophoretic mobility. Antibodies can also be used therapeutically, to decrease the specific activity of the serine racemase, as described below.

Antibodies which specifically bind to epitopes of mammalian serine racemase proteins, polypeptides, or fusion proteins can be used in immunochemical assays, including but not limited to Western blots, ELISAs, radioimmunoassays, immunohistochemical assays, immunoprecipitations, or other immunochemical assays known in the art provide a detection signal in immunoassays such as which is at least

5- , 10- , or 20-fold higher than a detection signal provided with other proteins when used in such immunochemical assays. Preferably, antibodies which specifically bind to mammalian serine racemase epitopes do not detect other proteins in immunochemical assays and can immunoprecipitate the enzyme or fragments thereof from solution.

Mammalian serine racemase-specific antibodies specifically bind to epitopes present in a mammalian serine racemase having an amino acid sequence encoded by polynucleotide molecules comprising the nucleotide sequences shown in SEQ ID NOS: 1, 2, 3, or 9. Typically, at least 6, 8, 10, or 12 contiguous amino acids are required to form an epitope. However, epitopes which involve non-contiguous amino acids may require more, *e.g.*, at least 15, 25, or 50 amino acids.

Epitopes of mammalian serine racemase which are particularly antigenic can be selected, for example, by routine screening of mammalian serine racemase polypeptides for antigenicity or by applying a theoretical method for selecting antigenic regions of the protein, using methods such as those taught in Harlow & Lane (1988), Hopp & Wood, *Proc. Natl. Acad. Sci. U.S.A.* 78, 3824-28 (1981), Hopp & Wood, *Mol. Immunol.* 20, 483-89 (1983), and Sutcliffe *et al.*, *Science* 219, 660-66 (1983).

Any type of antibody known in the art can be generated to bind specifically to mammalian serine racemase epitopes. For example, preparations of polyclonal and monoclonal antibodies can be made using standard methods which are well known in the art. Similarly, single-chain antibodies can also be prepared. Single-chain antibodies which specifically bind to mammalian serine racemase epitopes can be isolated, for example, from single-chain immunoglobulin display libraries, as is known in the art and described, for example, in Hayashi *et al.*, 1995, *Gene* 160: 129-30. Single-chain antibodies can also be constructed using a DNA amplification method, such as the polymerase chain reaction (PCR), using hybridoma cDNA as a template. Thirion *et al.*, 1996, *Eur. J. Cancer Prev.* 5: 507-11.

Single-chain antibodies can be mono- or bispecific, and can be bivalent or tetravalent. Construction of tetravalent, bispecific single-chain antibodies is taught, for example, in Coloma and Morrison, 1997, *Nat. Biotechnol.* 15: 159-63. Construction of bivalent, bispecific single-chain antibodies is taught *inter alia* in

Mallender and Voss, 1994, *J. Biol. Chem.* 269: 199-206.

A nucleotide sequence encoding a single-chain antibody can be constructed using manual or automated nucleotide synthesis, cloned into an expression construct using standard recombinant DNA methods, and introduced into a cell to express the coding sequence, as described below. Alternatively, single-chain antibodies can be produced directly using, for example, filamentous phage technology. Verhaar *et al.*, 1995, *Int. J. Cancer* 61: 497-501; Nicholls *et al.*, 1993, *J. Immunol. Meth.* 165: 81-91.

For use in therapeutic methods, monoclonal and other antibodies can be "humanized" in order to prevent a patient from mounting an immune response against the antibody. Such antibodies can be sufficiently similar in sequence to human antibodies to be used directly in therapy or may require alteration of a few key residues. Sequence differences between, for example, rodent antibodies and human sequences can be minimized by replacing residues which differ from those in the human sequences, for example, by site directed mutagenesis of individual residues, or by grafting of entire complementarity determining regions. Alternatively, one can produce humanized antibodies using recombinant methods, as described in GB2188638B. Antibodies which specifically bind to mammalian serine racemase epitopes can contain antigen binding sites which are either partially or fully humanized, as disclosed in U.S. 5,565,332.

Other types of antibodies can be constructed and used in methods of the invention. For example, chimeric antibodies can be constructed as disclosed, for example, in WO 93/03151. Binding proteins which are derived from immunoglobulins and which are multivalent and multispecific, such as the "diabodies" described in WO 94/13804, can also be prepared.

Antibodies of the invention can be purified by methods well known in the art. For example, antibodies can be affinity purified by passing the antibodies over a column to which a mammalian serine racemase protein, polypeptide, or fusion protein is bound. The bound antibodies can then be eluted from the column, using a buffer with a high salt concentration.

In one embodiment of the invention, serine racemase is inhibited by administering antibodies which specifically bind to serine racemase to a patient in need of such inhibition. Preparation of such antibodies is described above.

Coding sequences for mammalian serine racemase proteins are shown in SEQ ID NOS: 1, 2, 3, and 9. SEQ ID NO: 1 is the coding sequence for mouse serine racemase. SEQ ID NO: 9 comprises the coding sequence for mouse serine racemase. SEQ ID NOS: 2 and 3 encode the N- and C-termini of human serine racemase, respectively. Full-length cDNA encoding the human enzyme can be obtained using polynucleotide probes selected from SEQ ID NOS: 2, 3, or 9 to screen human cDNA using methods known in the art. Alternatively, human expression libraries can be screened for cDNA clones which express human serine racemase using specific antibodies of the invention.

Isolated and purified mammalian serine racemase polynucleotide molecules according to the invention are subgenomic and contain less than a whole chromosome. Preferably, the polynucleotides are intron-free. Isolated and purified polynucleotide molecules of the invention can be single-or double-stranded and can comprise at least 362, 400, 500, 600, 700, 800, 900, or 1000 contiguous nucleotides selected from SEQ ID NOS: 1 or 9 or can comprise SEQ ID NOS: 1, 2, 3, or 9.

Complements of the nucleotide sequences shown in SEQ ID NOS: 1, 2, 3, and 9 are contiguous nucleotide sequences which form Watson-Crick base pairs with a contiguous nucleotide sequence of SEQ ID NOS: 1, 2, 3, and 9. Complements of the nucleotide sequences shown in SEQ ID NOS: 1, 2, 3, and 9 are polynucleotides of the invention and can be used, for example, to provide antisense oligonucleotides, primers, and probes. Antisense oligonucleotides, primers, and probes of the invention can consist of at least 11, 12, 15, 20, 25, 30, 50, or 100 contiguous nucleotides which are complementary to the coding sequences shown in SEQ ID NOS: 1, 2, 3, and 9. A complement of the entire coding sequence can also be used.

Degenerate nucleotide sequences which encode amino acid sequences of mammalian serine racemase protein as well as homologous nucleotide sequences which are at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequences shown in SEQ ID NOS: 1, 2, 3, and 9, are also polynucleotides of the invention. Percent sequence identity is determined using computer programs which employ the Smith-Waterman algorithm, for example as implemented in the MPSRCH program (Oxford Molecular), using an affine gap search with the following parameters: a gap open penalty of 12 and a gap extension penalty of 1.

Nucleotide sequences which hybridize to the coding sequences shown in SEQ ID NOS: 1, 2, 3, or 9 or their complements with at most 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, or 35% basepair mismatches are also polynucleotides of the invention. For example, using the following wash conditions--2 x SSC (0.3 M sodium chloride, 0.03 M sodium citrate, pH 7.0), 0.1% SDS, room temperature twice, 30 minutes each; then 2 x SSC, 0.1% SDS, 50 °C once, 30 minutes; then 2 x SSC, room temperature twice, 10 minutes each--homologous polynucleotide sequences can be identified which contain at most about 25-30% basepair mismatches with SEQ ID NOS: 1, 2, 3, or 9. More preferably, homologous nucleic acid strands contain 15-25% basepair mismatches, even more preferably 5-15% basepair mismatches.

Other mammalian serine racemase-encoding polynucleotides can be identified by making suitable probes or primers and screening cDNA expression libraries from other mammals, such as monkey, pig, cow, sheep, goat, or guinea pig. Similarly, serine racemase enzymes from non-mammalian species, such as yeast and *Drosophila*, can be identified by screening cDNA expression libraries from these species using degenerate probes and primers based on the polynucleotide sequences disclosed herein. The  $T_m$  of a double-stranded DNA decreases by 1-1.5 °C with every 1% decrease in homology (Bonner *et al.*, *J. Mol. Biol.* 81, 123 (1973)). Homologous mammalian or non-mammalian serine racemase polynucleotides can therefore be identified, for example, by hybridizing a putative homologous polynucleotide with a polynucleotide having a nucleotide sequence as shown in SEQ ID NOS: 1, 2, 3, or 9, comparing the melting temperature of the test hybrid with the melting temperature of a hybrid comprising a polynucleotide having SEQ ID NOS: 1, 2, 3, or 9 and a polynucleotide which is perfectly complementary to that sequence, and calculating the number or percent of basepair mismatches within the test hybrid.

Nucleotide sequences which hybridize to the coding sequences shown in SEQ ID NOS: 1, 2, 3, or 9 under stringent hybridization and/or wash conditions are also polynucleotides of the invention. Stringent wash conditions are well known and understood in the art and are disclosed, for example, in Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL, 2d ed., 1989, at pages 9.50-9.51.

Typically, stringent hybridization conditions include a combination of temperature and salt concentration that is approximately 12-20 °C below the calculated

$T_m$  of the hybrid under study. The  $T_m$  of a hybrid between the polynucleotide sequences shown in SEQ ID NOS: 1, 2, 3, and 9 and a polynucleotide sequence which is 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical can be calculated, for example, using the equation of Bolton and McCarthy, *Proc. Natl. Acad. Sci. U.S.A.* 48, 1390 (1962):

$$T_m = 81.5\text{ }^{\circ}\text{C} - 16.6(\log_{10}[\text{Na}^+]) + 0.41(\%G + C) - 0.63(\%\text{formamide}) - 600/l,$$

where  $l$  = the length of the hybrid in basepairs.

Stringent wash conditions include, for example, 4 x SSC at 65 °C, or 50% formamide, 4 x SSC at 42 °C, or 0.5 x SSC, 0.1% SDS at 65 °C. Highly stringent wash conditions include, for example, 0.2 x SSC at 65 °C.

Polynucleotides of the invention can be isolated and purified free from other nucleotide sequences using standard nucleic acid purification techniques. For example, restriction enzymes and probes can be used to isolate polynucleotide fragments which comprise coding sequences for a mammalian serine racemase. Isolated and purified polynucleotides are in preparations which are free or at least 90% free of other molecules.

Complementary DNA (cDNA) molecules which encode mammalian serine racemase proteins are also polynucleotides of the invention. cDNA molecules can be made using standard molecular biology techniques, with mammalian serine racemase mRNA as a template. cDNA molecules can thereafter be replicated using molecular biology techniques known in the art and disclosed in manuals such as Sambrook *et al.*, 1989. An amplification technique, such as the polymerase chain reaction (PCR), can be used to obtain additional copies of polynucleotides of the invention, using either genomic DNA or cDNA as a template.

Alternatively, synthetic chemistry techniques can be used to synthesize polynucleotide molecules of the invention. The degeneracy of the genetic code allows alternate nucleotide sequences to be synthesized which will encode mammalian serine racemase proteins. All such nucleotide sequences are within the scope of the present invention.

The invention also provides polynucleotide probes which can be used to detect sequences which encode mammalian serine racemase, for example, in hybridization protocols such as Northern or Southern blotting or *in situ* hybridization.

Polynucleotide probes of the invention comprise at least 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, or 40 or more contiguous nucleotides selected from SEQ ID NOS: 1, 2, 3, or 9. Polynucleotide probes of the invention can comprise a detectable label, such as a radioisotopic, fluorescent, enzymatic, or chemiluminescent label.

5           Using recombinant DNA techniques, a polynucleotide of the present invention can be ligated together with other polynucleotide sequences to form an expression construct. Such expression constructs can be used to express all or a portion of a mammalian serine racemase, such as a mouse, rat, or human serine racemase, in a host cell. An expression construct of the invention comprises a polynucleotide segment  
10           encoding the desired amino acid sequence and a promoter which is functional in the particular host cell selected. The skilled artisan can readily select an appropriate promoter from the large number of cell type-specific promoters known and used in the art. The polynucleotide segment is located downstream from the promoter. Transcription of the polynucleotide segment initiates at the promoter. An expression  
15           construct can also contain a transcription terminator which is functional in the host cell. The expression construct can be linear or circular and can contain sequences, if desired, for autonomous replication.

          The host cell comprising the expression construct can be prokaryotic or eukaryotic. A variety of host cells for use in mammalian, yeast, bacterial, or insect  
20           expression systems are available and can be used to express the construct. Suitable mammalian host cells include, for example, monkey COS cells, Chinese Hamster Ovary (CHO) cells, human kidney 293 cells, human epidermal A431 cells, human Colo205 cells, 3T3 cells, CV-1 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from *in vitro* culture of primary tissue, primary  
25           explants, HeLa cells, mouse L cells, baby hamster kidney cells, HL-60, U937, HaK, or Jurkat cells.

          Yeast or prokaryotic host cells can also be used to produce mammalian serine racemase. Suitable yeast strains include *Saccharomyces cerevisiae*,  
30           *Schizosaccharomyces pombe*, *Kluyveromyces* strains, *Candida*, or any yeast strain capable of expressing heterologous proteins. Suitable bacterial strains include *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhimurium*, or any bacterial strain capable of expressing heterologous proteins. If the protein is made in yeast or

bacteria, it may be necessary to modify it using known chemical or enzymatic methods in order to obtain a functional racemase. Such modification techniques are well known in the art.

Mammalian serine racemase can also be produced in an insect expression system. Materials and methods for baculovirus/insect cell expression systems, for example, are commercially available in kit form from, *e.g.*, Invitrogen, San Diego, Calif., U.S.A. (the MaxBat Registered TM kit), and such methods are well known in the art, as described in Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987).

According to one embodiment of the invention, mammalian serine racemase is produced by culturing a host cell which comprises an isolated and purified polynucleotide molecule which encodes the racemase under culture conditions suitable to express the recombinant protein. The resulting expressed protein can then be purified from either the culture medium or the host cells using known techniques such as those disclosed in Example 2.

The racemase can be expressed in a form which will facilitate purification. For example, it can be expressed as a fusion protein, such as those of maltose binding protein (MBP), glutathione-S-transferase (GST) or thioredoxin (TRX). Kits for expression and purification of such fusion proteins are commercially available from New England BioLab (Beverly, Mass.), Pharmacia (Piscataway, N.J.) and In Vitrogen, respectively. The protein can also be tagged with an epitope and subsequently purified by using a specific antibody directed to the epitope. One such epitope ("Flag") is commercially available from Kodak (New Haven, Conn.). Monoclonal antibodies which recognize the "Flag" epitope are also commercially available (Kodak Scientific Imaging Systems).

Expression constructs can be introduced into the host cells using any technique known in the art. These techniques include transferrin-polycation-mediated DNA transfer, transfection with naked or encapsulated nucleic acids, liposome-mediated cellular fusion, intracellular transportation of DNA-coated latex beads, protoplast fusion, viral infection, electroporation, "gene gun," and calcium phosphate-mediated transfection.

Viral-based vectors can be used to introduce expression constructs of the

invention into host cells. Recombinant retroviruses can be used, as described for example in Mann *et al.*, *Cell* 33: 153, 1983, Cane and Mulligan, *Proc. Natl. Acad. Sci. USA* 81: 6349, 1984, and Miller *et al.*, *Human Gene Therapy* 1: 5-14, 1990, U.S. Patent Nos. 4,405,712, 4,861,719, and 4,980,289; and PCT Application Nos. WO 89/02,468, WO 89/05,349, and WO 90/02,806. Recombinant adenoviral vectors can also be prepared and used, given the disclosure provided herein (*see* Berkner, *Biotechniques* 6: 616, 1988, and Rosenfeld *et al.*, *Science* 252: 431, 1991, WO 93/07283, WO 93/06223, and WO 93/07282). Adeno-associated viral vectors can also be used to deliver polynucleotides of the invention to host cells. The use of adeno-associated viral vectors *in vitro* is described in Chatterjee *et al.*, *Science* 258: 1485-1488 (1992), Walsh *et al.*, *Proc. Natl. Acad. Sci.* 89: 7257-7261 (1992), Walsh *et al.*, *J. Clin. Invest.* 94: 1440-1448 (1994), Flotte *et al.*, *J. Biol. Chem.* 268: 3781-3790 (1993), Ponnazhagan *et al.*, *J. Exp. Med.* 179: 733-738 (1994), or Miller *et al.*, *Proc. Natl. Acad. Sci.* 91: 10183-10187 (1994), Einerhand *et al.*, *Gene Ther.* 2: 336-343 (1995), Luo *et al.*, *Exp. Hematol.* 23: 1261-1267 (1995), and Zhou *et al.*, *Gene Therapy* 3: 223-229 (1996). *In vivo* use of these vehicles is described in Flotte *et al.*, *Proc. Natl. Acad. Sci.* 90: 10613-10617 (1993), and Kaplitt *et al.*, *Nature Genet.* 8: 148-153 (1994).

Other viruses which can be used to construct vectors include herpes simplex virus (Kit *et al.*, *Adv. Exp. Med. Biol.* 215: 219, 1989) (ATCC VR-977; ATCC VR-260); *Nature* 277: 108, 1979), human immunodeficiency virus (EPO 386,882, Buchschacher *et al.*, *J. Vir.* 66: 2731, 1992), and measles virus (EPO 440,219) (ATCC VR-24); A (ATCC VR-67; ATCC VR-1247). Any suitable vector known in the art can be used to introduce polynucleotides or expression constructs of the invention into host cells.

Endogenous D-serine is required for physiologic NMDA neurotransmission. Treatment of brain slices or cultures with D-amino acid oxidase, under conditions in which D-serine is completely degraded, greatly reduces NMDA transmission, whether measured neurophysiologically or by measuring stimulation of nitric oxide synthase activity or levels of cyclic GMP. Drugs which inhibit mammalian serine racemase can be used to treat conditions or diseases in which NMDA overexcitation is found. In particular, such conditions include those for which NMDA receptor antagonists

have displayed efficacy.

For example, activation of NMDA receptors is an important pathologic event in stroke and several neurodegenerative diseases. Inhibitors of serine racemase can be used to decrease D-serine levels in the brain and consequently decrease the activation of NMDA receptors. Identification of agents which inhibit serine racemase is therefore important in the treatment of any disease that includes acute or chronic neuronal death or dysfunction mediated by overactivation of NMDA receptors, such as stroke, epilepsy, and chronic neurodegenerative diseases such as Parkinson's disease, Huntington's disease, motor neuron diseases, and Alzheimer's disease.

In one embodiment of the invention serine racemase is inhibited using an antisense oligonucleotide. The sequence of the antisense oligonucleotide is complementary to at least a portion of a coding sequence as shown in SEQ ID NOS: 1, 2, 3, or 9. Preferably, the antisense oligonucleotide is at least six nucleotides in length, but can be at least 8, 11, 12, 15, 20, 25, 30, 35, 40, 45, or 50 nucleotides long. Longer sequences can also be used.

Antisense oligonucleotides can be composed of deoxyribonucleotides, ribonucleotides, or a combination of both. Oligonucleotides can be synthesized manually or by an automated synthesizer, by covalently linking the 5' end of one nucleotide with the 3' end of another nucleotide with non-phosphodiester internucleotide linkages such as alkylphosphonates, phosphorothioates, phosphorodithioates, alkylphosphonothioates, alkylphosphonates, phosphoramidates, phosphate esters, carbamates, acetamidate, carboxymethyl esters, carbonates, and phosphate triesters. See Brown, 1994, *Meth. Mol. Biol.* 20: 1-8; Sonveaux, 1994, *Meth. Mol. Biol.* 26: 1-72; Uhlmann *et al.*, 1990, *Chem. Rev.* 90: 543-583.

Although desirable, precise complementarity is not required for successful duplex formation between an antisense molecule and the complementary coding sequence of a polynucleotide which encodes a mammalian serine racemase. Antisense molecules which comprise, for example, 2, 3, 4, or 5 or more stretches of contiguous nucleotides which are precisely complementary to a mammalian serine racemase coding sequence, each separated by a stretch of contiguous nucleotides which are not complementary to adjacent coding sequences, can provide sufficient targeting specificity for mammalian serine racemase mRNA. Preferably, each stretch of

contiguous nucleotides is at least 4, 5, 6, 7, or 8 or more nucleotides in length. Non-complementary intervening sequences are preferably 1, 2, 3, or 4 nucleotides in length. One skilled in the art can easily use the calculated melting point of an antisense-sense pair to determine the degree of mismatching which will be tolerated between a particular antisense oligonucleotide and a particular coding sequence.

Antisense oligonucleotides can be modified without affecting their ability to hybridize to a mammalian serine racemase coding sequence. These modifications can be internal or at one or both ends of the antisense oligonucleotide. For example, internucleoside phosphate linkages can be modified by adding cholesteryl or diamine moieties with varying numbers of carbon residues between the amino groups and terminal ribose. Modified bases and/or sugars, such as arabinose instead of ribose, or a 3', 5'-substituted oligonucleotide in which the 3' hydroxyl group or the 5' phosphate group are substituted, can also be used in a modified antisense oligonucleotide. These modified oligonucleotides can be prepared by methods well known in the art. Agrawal *et al.*, *Trends Biotechnol.* 10: 152-158, 1992; Uhlmann *et al.*, *Chem. Rev.* 90: 543-584, 1990; Uhlmann *et al.*, *Tetrahedron. Lett.* 215: 3539-3542, 1987.

Therapeutic compositions of the invention can also comprise a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers are well known to those in the art. Such carriers include, but are not limited to, large, slowly metabolized macromolecules, such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and inactive virus particles. Pharmaceutically acceptable salts can also be used, for example, mineral salts such as hydrochlorides, hydrobromides, phosphates, or sulfates, as well as the salts of organic acids such as acetates, propionates, malonates, or benzoates.

Therapeutic compositions can also contain liquids, such as water, saline, glycerol, and ethanol, as well as substances such as wetting agents, emulsifying agents, or pH buffering agents. Liposomes, such as those described in U.S. 5,422,120, WO 95/13796, WO 91/14445, or EP 524,968 B1, can also be used as a carrier for a therapeutic composition. Typically, a therapeutic composition of the invention is prepared as an injectable, either as a liquid solution or suspension; however, solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared.

Administration of antisense oligonucleotides or antibodies of the invention can include local or systemic administration, including injection, oral administration, particle gun, or catheterized administration. Various methods can be used to administer a therapeutic composition directly to a specific site in the body. For example, receptor-mediated targeted delivery can be used to deliver therapeutic compositions containing antisense oligonucleotides or antibodies to specific tissues. Receptor-mediated delivery techniques are described, for example, in Findeis *et al.* (1993) *Trends in Biotechnol.* 11, 202-05; Chiou *et al.* (1994), GENE THERAPEUTICS: METHODS AND APPLICATIONS OF DIRECT GENE TRANSFER (J.A. Wolff, ed.); Wu & Wu (1988), *J. Biol. Chem.* 263, 621-24; Wu *et al.* (1994), *J. Biol. Chem.* 269, 542-46; Zenke *et al.* (1990), *Proc. Natl. Acad. Sci. U.S.A.* 87, 3655-59; Wu *et al.* (1991), *J. Biol. Chem.* 266, 338-42.

If the composition contains antibodies, effective dosages of the composition are in the range of about 5 µg to about 50 µg/kg of patient body weight, about 50 µg to about 5 mg/kg, about 100 µg to about 500 µg/kg of patient body weight, and about 200 to about 250 µg/kg. Therapeutic compositions containing antisense oligonucleotides can be administered in a range of about 100 ng to about 200 mg, about 500 ng to about 50 mg, about 1 µg to about 2 mg, about 5 µg to about 500 µg, or about 20 µg to about 100 µg. Factors such as method of action and efficacy of transformation and expression are considerations that will effect the dosage required for ultimate efficacy of therapeutic compositions of the invention. Where greater expression is desired over a larger area of tissue, larger amounts of therapeutic compositions or the same amounts readministered in successive administrations can be used to effect a positive therapeutic outcome. In all cases, routine experimentation in clinical trials will determine specific ranges for optimal therapeutic effect.

The invention also provides a method of screening test compounds to identify candidate therapeutic agents which modulate the activity of mammalian serine racemase. Modulators can either increase or decrease the specific activity of mammalian serine racemase. A test compound can be a pharmacologic agent already known in the art or can be a compound previously unknown to have any pharmacological activity. The compound can be naturally occurring or designed in the laboratory. It can be isolated from microorganisms, animals, or plants, and can be

produced recombinantly, or synthesized by chemical methods known in the art.

A test compound is contacted with a mammalian serine racemase, such as a rat, mouse, or human serine racemase. The activity of the serine racemase can be assayed as described in Example 2. Any other suitable assay for serine racemase activity can also be used.

A test compound is identified as a candidate therapeutic agent if it modulates the activity of the mammalian serine racemase. Preferably the activity of the mammalian serine racemase is increased or decreased by at least 50%, 60%, 70%, or 80%. Most preferably, activity is increased or decreased by 90%, 95%, 99%, or 100%.

The complete contents of all references cited in this disclosure are incorporated herein by reference. The following examples are provided for exemplification purposes only and are not intended to limit the scope of the invention which has been described in broad terms above.

## EXAMPLES

*The following materials were used in the examples below.*

Amino acids, aminooxyacetic acid (AOAA), catalase, oxidized glutathione, hydroxylamine, leupeptin, luminol (sodium salt), pepstatin, phenylmethylsulfonyl fluoride, pyridoxal 5'-phosphate (PLP), o-phthaldialdehyde (OPA), L-homocysteic acid, and Tris were obtained from Sigma (St. Louis). D-amino acid oxidase from pig kidney (EC 1.4.3.3), dithiothreitol (DTT), and horseradish peroxidase were obtained from Boehringer Mannheim. Ammonium sulfate and  $\text{KH}_2\text{PO}_4$  were purchased from J.T. Baker. Butyl sepharose 4 fast flow, Q-sepharose, mono Q HR 5/5 were obtained from Pharmacia. Macro-prep ceramic hydroxyapatite type I (20  $\mu\text{M}$ ) was purchased from Bio-Rad. N-tert-butyloxycarbonyl-L-cysteine (L-Boc-cys) was obtained from Novabiochem. Other reagents were of analytical grade.

## EXAMPLE 1

*This example demonstrates purification of mammalian serine racemase.*

Sixty brains from 10-14 day old Sprague-Dawley rats were homogenized using a Polytron in 5 volumes of ice-cold buffer A (10 mM KPi, pH 7.2, 50 mM KCl, 1 mM

EDTA, 2 mM DTT, 15  $\mu$ M PLP, 0.2 mM freshly prepared phenylmethylsulfonyl fluoride, 1  $\mu$ g/ml leupeptin, 1  $\mu$ g/ml pepstatin). All subsequent steps were performed at 4 °C. The homogenate was centrifuged at 40,000 x g for 20 minutes, and the supernatant was brought to 45% ammonium sulfate saturation under continuous stirring. After a 40 minutes precipitation, the solution was centrifuged at 20,000 x g for 20 minutes. The pellet was resuspended in 20% ammonium sulfate in buffer A. The suspension was left on ice for 1 hour and then centrifuged at 20,000 x g for 20 minutes to remove insoluble aggregates.

The supernatant was loaded at 3 ml/minute onto a 70 ml butyl-sepharose column pre-equilibrated with 20% ammonium sulfate in buffer A. The column was washed with 210 ml of 10% ammonium sulfate, and the active fraction was eluted with 5% ammonium sulfate in buffer A. The eluted material was concentrated by precipitation with 50% ammonium sulfate and centrifugation at 20,000 x g for 20 min. The pellet was resuspended in 6-8 ml buffer A and dialyzed overnight against 4 liters of buffer B (10 mM KPi, pH 7.2, 50 mM KCl, 1 mM EDTA, 2 mM DTT, 15  $\mu$ M PLP). After dialysis, the suspension was centrifuged at 20,000 x g for 20 minutes to remove insoluble aggregates, and the supernatant was loaded at 0.5 ml/ml onto a 3 ml Q-sepharose column.

After washing with 10 ml loading buffer, the protein was eluted with 250 mM NaCl in buffer B. The eluted material was concentrated with centriprep 30 (Amicon, Lexington, Mass.) and diluted in buffer B without KCl to decrease the salt concentration to 50 mM. Then, the suspension was loaded at 0.5 ml/minutes onto mono Q column. The column was washed with buffer B containing 150 mM KCl, and the protein was eluted with a linear gradient of KCl in the range of 158 to 188 mM KCl.

The active fractions were pooled, concentrated with centriplus 30 (Amicon, Lexington, Mass) and diluted in buffer B without EDTA and KPi. The procedure was repeated once to decrease the EDTA and KPi concentrations to 20  $\mu$ M and 0.75 mM, respectively. To this suspension,  $\text{CaCl}_2$  was added (300  $\mu$ M final concentration) to improve the protein binding to hydroxyapatite. The protein was applied at 0.1 ml/minutes to a 1 ml hydroxyapatite column, and eluted with a linear gradient of 0.75 to 400 mM KPi in buffer containing 50 mM KCl, 2 mM DTT and 15  $\mu$ M PLP. The

purified protein was typically eluted in the range of 0.75 to 30 mM Kpi.

For most applications the purified protein was further concentrated using centriplus 30. Protein concentration was determined with Coomassie plus protein assay reagent (Pierce). Enzyme-bound PLP was determined at room temperature by recording the absorbance of the purified protein in the range of 500 to 280 nm using a Lambda Bio spectrophotometer (Perkin Elmer). SDS gel electrophoresis revealed a single band for the purified protein of about 37 kDa (Figure 1).

We have therefore purified the enzyme to homogeneity utilizing sequentially ammonium sulfate fractionation, butyl-sepharose, Q-sepharose, mono-Q, and hydroxyapatite chromatography steps (Table 1). The overall purification from the ammonium sulfate fraction is 12,500. Enzyme activity is obtained in 30% yield. Interestingly, there is an apparent increase in yield following the Q-sepharose step, suggesting the removal of an inhibitor.

Serine racemase is a relatively small soluble protein of 37 kDa. Its absorption spectrum indicates that no minor, undetected protein could account for enzyme activity. The magnitude and ratios of absorption at 280, 340, and 420 nm closely resemble values for known PLP enzymes (20, 23, 24). This could only be possible if essentially all the protein is the PLP-requiring racemase.

The enzyme is stable with no loss of activity when stored for 4 days at 4 °C. Only modest loss of activity occurs after two cycles of freezing and thawing. The enzyme appears to be soluble with no activity detected in a membrane preparation. Enzyme activity displays a sharp pH optimum in the alkaline range, with optimal activity at pH 8-9 being about 10 times higher than at pH 7 (Figure 2). Enzyme activity is maximal at 37 °C, and is abolished by boiling.

## **EXAMPLE 2**

*This example demonstrates an assay of serine racemase activity.*

D-serine formation was monitored by a chemiluminescent assay that specifically detects D-serine. Racemase activity was performed in the presence of 50 mM Tris-HCl (pH 8.0), 18 µl enzyme extract, 1 mM EDTA, 2 mM DTT, 15 µM pyridoxal 5'-phosphate (PLP), and 20 mM L-serine. After 0.5 to 8 hours incubation at 37 °C, the reaction was terminated by the addition of trichloroacetic acid (TCA) to

a final concentration of 5%. Blanks employed boiled enzyme extract. The precipitated protein was removed by centrifugation, and the supernatant was extracted 2 times with 1 ml of water-saturated diethyl ether to remove TCA. D-serine concentration was determined by incubation of the samples with D-amino acid oxidase, which specifically degrades D-amino acids, generating an  $\alpha$ -keto acid,  $\text{NH}_3$ , and hydrogen peroxide (16).

The generation of hydrogen peroxide was quantitated by the use of peroxidase and luminol, which emits light. A 10  $\mu\text{l}$  sample aliquot was added to 100  $\mu\text{l}$  of medium containing 100 mM Tris-HCl (pH 8.8), 10 U/ml peroxidase, and 8  $\mu\text{M}$  luminol. After a 10 to 20 minute delay required to decrease the nonspecific luminol luminescence, 10  $\mu\text{l}$  of D-amino acid oxidase (75 U/ml) were added, and the tubes were gently mixed with a pipette tip. Maximum luminescence was recorded after 10 to 15 minutes at room temperature using a Monolight 2010 luminometer (Analytical Luminescence Laboratory). The amount of D-serine in each sample was calculated by comparison with standard curves. The measurements were reliable in the range of 50 to 2000 pmol D-serine per sample. Addition of mM concentrations of L-serine did not alter the values measured for D-serine. Alternatively, amino acid enantiomers were separated by high performance liquid chromatography (HPLC) using a carbon 18 reverse phase column (RP18 Spheri-5, 22 cm x 4.6 mm, Perkin Elmer) with fluorimetric detection after derivatization with L-Boc-cys and OPA, as described (17). The results obtained with chemiluminescent assay were identical to those obtained using HPLC.

The presence of trace amounts of D-serine in the commercial L-serine reagent generates high blank values. Thus, the stock solution of L-serine (100 mM) was routinely pre-treated for 3 days with 30 units of D-amino acid oxidase and 500 units of catalase to remove any D-serine contaminant. The enzymes were precipitated by the addition of 5% TCA. After removal of TCA by extraction with diethyl ether, the L-serine solution was neutralized with NaOH and could be used without further purification, being virtually free of any D-serine contaminant. The same purification procedure was applied for L-alanine and L-threonine.

**EXAMPLE 3**

*This example demonstrates that mammalian serine racemase activity obeys Michaelis-Menten kinetics (Figure 3).*

Monitoring the conversion of L to D-serine, the  $K_m$  is about 10 mM with a  
5  $V_{max}$  of 5  $\mu\text{mol/mg/h}$ . The enzyme can also convert D- to L-serine but with lesser  
affinity: the  $K_m$  in this direction is 60 mM, though the  $V_{max}$  is higher, 22  $\mu\text{mol/mg/h}$ .

**EXAMPLE 4**

10 *This example demonstrates that mammalian serine racemase requires pyridoxal 5'-phosphate (PLP).*

Dialysis for 16 hours against 1,000 volumes of the purification buffer without  
PLP abolishes enzyme activity, which can be restored by the addition of PLP.  
Aminooxyacetic acid (AOAA) and hydroxylamine, which inactivate PLP, inhibit  
enzyme activity (Figure 4). Examination of the absorption spectrum of the enzyme  
15 confirms the importance of PLP. Thus, the normal enzyme preparation displays  
absorption peaks at 420 nm and 340 nm, characteristic of PLP-dependent enzymes.  
The peak at 420 nm, corresponding to a Schiff's base complex of PLP with an active  
site lysine, is abolished by treatment with AOAA (Figure 5).

Sulfhydryl groups seem to be important for enzyme activity, as oxidized  
20 glutathione markedly reduces enzyme activity (Figure 4). We wondered whether  
conversion of L to D-serine might be a by-product of a different enzyme activity with  
non-enzymatic racemization giving rise to D-serine. Accordingly, we monitored by  
HPLC levels of L and D-serine at different incubation times (Table 2). Increases in  
formation of D-serine are paralleled by stoichiometric decreases in levels of L-serine,  
25 making it unlikely that L-serine is converted to any other compound by the enzyme.

Additionally, we examined the purified enzyme for the presence of other  
enzyme activities which might indirectly contribute to D-serine formation. We found  
no evidence for serine: pyruvate aminotransferase activity, as L-serine levels were not  
altered in the presence of pyruvate. We did not observe serine  
30 hydroxymethyltransferase activity inasmuch as we fail to detect the formation of  
glycine after extensive incubation of the enzyme with L-serine. There is no evidence  
for serine dehydratase activity which would be associated with a marked decrease in

L-serine levels. Serine racemase is highly selective for L-serine (Table 3). The enzyme displays about 1.5% as much activity toward L-alanine as for L-serine, while no activity is demonstrated with L-threonine or L-aspartate.

#### 5 **EXAMPLE 5**

*This example demonstrates cloning of mammalian serine racemase.*

Purified rat serine racemase was submitted to internal peptide sequencing and the following amino acid peptides were identified:

LLIEPTAGVGLAAVLSQHFQTVSPEVK (SEQ ID NO: 6)

10 HLNIQDSVHLTPVLTSSILNQLAGR (SEQ ID NO: 7).

Blast search using the blastp program did not reveal any protein with significant homology to the peptides.

We performed a blast search using the tblastn program against the EST database and found several independent ESTs sequences with unknown function that covered the N-terminal and the C-terminal regions of the protein (Accession numbers  
15 AA170919 (mouse), AI173393, AA034539, W89934, AA509764, AA833469). To obtain full length cDNA clones, we designed the following PCR primers based on the EST sequences we found in the database, containing restriction sites for *SalI* and *NotI*:

5' ACGCGTCGACCACCATGTGTGCTCAGTACTGC 3' (SEQ ID NO: 4) and  
20 5' ATAAGAATGCGGCCGCTTAAACAGAAACCGTCTG 3' (SEQ ID NO: 5).

Full length cDNA encoding mouse serine racemase was cloned by PCR from mouse brain cDNA obtained by reverse transcription of poly A RNA purchased from Clontech (Palo Alto, CA). The coding sequence is shown in SEQ ID NO: 1. The corresponding deduced amino acid sequence is shown in SEQ ID NO: 5.

25

#### **EXAMPLE 6**

*This example demonstrates expression of mammalian serine racemase.*

Serine racemase was cloned on mammalian expression vector PRK5 in *SalI/NotI*. HEK 293 cells were transfected with the full length racemase using the calcium chloride method. The cell were cultured Dulbecco's modified Eagles Medium supplemented with penicillin and streptomycin as described in Sawa *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 94, 11669-74 (1997). The medium was supplemented with 10  
30

mM L-serine for measurement of D-serine production. Culture medium and cell pellets were analyzed 48 hours after transfection for the presence of D-serine as described above.

Transfection of cells with cDNA encoding full-length serine racemase promoted a highly significant increase in D-serine concentration both in the medium and in the cell pellets (Figure 6). Cells transfected with vector alone showed only traces of D-serine.

### **EXAMPLE 7**

*This example demonstrates the deduction of the coding sequence of human serine racemase.*

Based on the mouse sequence, we searched the public database and found several human ESTS of unknown function with high homology to the mouse sequence. We generated a partial sequence of the human serine racemase gene by alignment of 11 of the ESTS. The partial sequence comprises the following contigs:

Contig 1 (SEQ ID NO: 2)

```
GGCGCGGCGCCGATGAGCTGAGAACCATGTGTGCTCAGTATTGCATCT
CCTTTGCTGATGTTGAAAAAGCTCATATCAACATTCGAGATTCTATCCA
CCTCACACCAGTGCTAACAAGCTCCATTTTGAATCAACTAACAGGGCGC
AATCTTTTCTTCAAATGTGAACTCTTCCAGAAAACAGGATCTTTTAAGA
TTCGTGGTGCTCTCAATGCCGTCAGAAGCTTGGTTCCTGATGCTTTAGA
AAGGAAGCCGAAAGCTGTTGTTACTCACAGCAGTGGAAACCATGGCCA
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GTGCCCCAGACAGCTCCAGACTGTAAAAAACTTGCAATACAAGCCTAC
GGAGCGTCAATTGTATACTGTGAACCTAGTGATGAAGTCCAGAGAAAA
TGTTGCAAAAAGGAGTTACAGAAGAAACAGAAGGCATCATGGTACATC
CCAACCAGGAACCTGCAGTGATAGCTGGACAAGGGACAATTGCCCTGG
AAGTGCTGAACCAGGTTTCTTTGGTGGATCCACTGGTGGNCCCTGTAGG
TGGAAGGAGGAATGCTTGCCGGGAAT
```

Contig 2 (SEQ ID NO: 3)

CTGATGCCCAATCTTTATCCTCCAGAAACCATAGCAGATGGTGTCAAA  
TCCAGCATTGGCTTGAANCACCTGGCCTATTATCAGGGACCTTGTGGATG  
ATATCTTCACTGTACAGAGGATGAAATTAAGTGTGCAACCCAGCTGGTG  
5 TGGGAGAGGATGAACTACTCATTGAACCTACAGCTGGTGTGGAGTGGC  
TGCTGTGCTGTCTCAACATTTTCAAACCTGTTTCCCCAGAAGTAAAGAACA  
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10 CATTTTGTTCCTAGTATTGTCAACTCTTAGTTATCAGATTCTTAATGGA  
GAGTGGCTATTCATTAAGGTTTAATAGTTTTTTTTTGGACTAAGTAGTGG  
AAAAACTTTTA

Contig 1 represents the N-terminal of human serine racemase and Contig 2  
15 represents the C-terminal as analyzed by standard DNA alignment program.

Table 1. Purification of serine racemase.

Fraction	Protein (mg)	Specific activity ( $\mu\text{mol L-ser/mg/h}$ )	Fold purification	Total activity	Yield (%)
Homogenate	4744	N.D. <sup>a</sup>	-	-	-
$(\text{NH}_4)_2\text{SO}_4$ fractionation	624	0.0004	1	0.249	100
Butyl-sepharose	34	0.003	7.5	0.102	41
Q-sepharose	6.6	0.029	73	0.191	77
Mono-Q	0.22	0.833	2082	0.183	73
Hydroxyapatite	0.015	5.0	12500	0.075	30

Enzyme was purified and fractions were assayed as described. Data represent a typical purification, which was repeated six times with similar results. <sup>a</sup> Not detected

Table 2. Racemization of L- serine

Time	[L-serine] (mM)	[D-serine] (mM)
0	4.00	0.0007
0.5 h	3.96	0.042
1h	3.93	0.101
4h	3.65	0.342

Racemase activity was assayed at 37 °C in a medium containing 50 mM Tris-HCl (pH 8.0), 4 mM L-serine, 40  $\mu\text{g/ml}$  purified enzyme, 1 mM EDTA, 2 mM DTT and 15  $\mu\text{M}$  pyridoxal 5'-phosphate. Samples were analyzed for amino acids enantiomers by HPLC as described.

Table 3. Substrate specificity of serine racemase

Amino acid	Specific activity ( $\mu\text{mol}/\text{mg}/\text{h}$ )	% control
L-serine	4.8	100
L-alanine	0.012	1.5
L-threonine	0	0
L-aspartate	0	0

Racemase activity was assayed at 37 °C in a medium containing 50 mM Tris-HCl (pH 8.0), 20 mM L-amino acids, 40-100  $\mu\text{g}/\text{ml}$  purified enzyme, 1 mM EDTA, 2 mM DTT and 15  $\mu\text{M}$  pyridoxal 5'-phosphate. After 8 h, the reaction was terminated by the addition of 5% TCA, and samples were analyzed by HPLC as described. The data represent a typical experiment which was replicated three times using different preparations with similar results.

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**CLAIMS**

1. A preparation of an isolated mammalian serine racemase having a specific activity of at least 0.003  $\mu$ mole L-serine/mg/hour.
2. The preparation of claim 1 wherein the specific activity is at least 0.025  $\mu$ mole L-serine/mg/hour.
3. The preparation of claim 1 wherein the specific activity is at least 0.075  $\mu$ mole L-serine/mg/hour.
4. The preparation of claim 1 wherein the specific activity is at least 1  $\mu$ mole L-serine/mg/hour.
5. The preparation of claim 1 wherein the specific activity is at least 2.5  $\mu$ mole L-serine/mg/hour.
6. The preparation of claim 1 wherein the specific activity is at least 5  $\mu$ mole L-serine/mg/hour.
7. The preparation of claim 1 wherein the racemase has an amino acid sequence as shown in SEQ ID NO: 8.
8. The preparation of claim 1 wherein the racemase has an amino acid sequence as shown in SEQ ID NO: 10.
9. The preparation of claim 1 wherein the racemase is murine.
10. The preparation of claim 1 wherein the racemase is rat.
11. The preparation of claim 1 wherein the racemase is human.
12. The preparation of claim 1 wherein the racemase comprises an amino acid sequence as shown in SEQ ID NO: 6, 7, 8, or 10.
13. A preparation of an isolated mammalian serine racemase having a specific activity of at least 0.003  $\mu$ mole L-serine/mg/hour wherein the racemase has a sequence selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 10, and sequences which are at least 85% identical to SEQ ID NOS: 8 or 10 as determined according to the Smith-Waterman homology search algorithm, using an affine gap search with gap open penalty of 12 and a gap extension penalty of 1.
14. An isolated and purified polynucleotide molecule which encodes a mammalian serine racemase.
15. The polynucleotide molecule of claim 14 consisting of a coding sequence for

mammalian serine racemase.

16. The polynucleotide molecule of claim 14 comprising a nucleotide sequence as shown in SEQ ID NO: 1.
17. The polynucleotide molecule of claim 14 comprising a nucleotide sequence as shown in SEQ ID NO: 2.
18. The polynucleotide molecule of claim 14 comprising a nucleotide sequence as shown in SEQ ID NO: 3.
19. The polynucleotide molecule of claim 14 comprising a nucleotide sequence as shown in SEQ ID NO: 9.
20. An isolated and purified polynucleotide molecule which encodes a mammalian serine racemase which is at least 85% identical a polynucleotide having a coding sequence as shown in SEQ ID NOS: 1, 2, 3, or 9 as determined according to the Smith-Waterman homology search algorithm, using an affine gap search with gap open penalty of 12 and a gap extension penalty of 1.
21. An expression vector comprising a polynucleotide molecule according to claim 14.
22. An expression vector comprising a polynucleotide molecule according to claim 19.
23. An expression vector comprising a polynucleotide molecule according to claim 20.
24. A host cell comprising an expression construct which comprises a polynucleotide sequence encoding a mammalian serine racemase.
25. A host cell comprising an expression construct which comprises a polynucleotide sequence as shown in SEQ ID NO: 1.
26. A host cell comprising an expression construct which comprises a polynucleotide sequence as shown in SEQ ID NO: 9.
27. A host cell comprising an expression construct which comprises a polynucleotide according to claim 20.
28. A method of producing a mammalian serine racemase comprising the steps of:  
culturing a host cell according to claim 24 in a culture medium;  
recovering a mammalian serine racemase from the culture medium or the host cell.

29. A method of producing a mammalian serine racemase comprising the steps of:

culturing a host cell according to claim 25 in a culture medium;  
recovering a mammalian serine racemase from the culture medium or  
the host cell.

30. A method of producing a mammalian serine racemase comprising the steps of:

culturing a host cell according to claim 26 in a culture medium;  
recovering a mammalian serine racemase from the culture medium or  
the host cell.

31. A method of producing a mammalian serine racemase comprising the steps of:

culturing a host cell according to claim 27 in a culture medium;  
recovering a mammalian serine racemase from the culture medium or  
the host cell.

32. A method to screen compounds to identify candidate therapeutic agents comprising the steps of:

contacting a test compound with a mammalian serine racemase;  
assaying activity of the mammalian serine racemase;

identifying a test compound as a candidate therapeutic agent if it  
modulates the activity of the mammalian serine racemase.

33. The method of claim 32 wherein the candidate therapeutic agent inhibits the activity of the mammalian serine racemase.

34. The method of claim 32 wherein the candidate therapeutic agent increases the activity of the mammalian serine racemase.

35. The method of claim 32 wherein the mammalian serine racemase is murine.

36. The method of claim 32 wherein the mammalian serine racemase is rat.

37. The method of claim 32 wherein the mammalian serine racemase is human.

38. The method of claim 32 wherein the mammalian serine racemase has a specific activity of at least 0.003  $\mu$ mole L-serine/mg/hour.

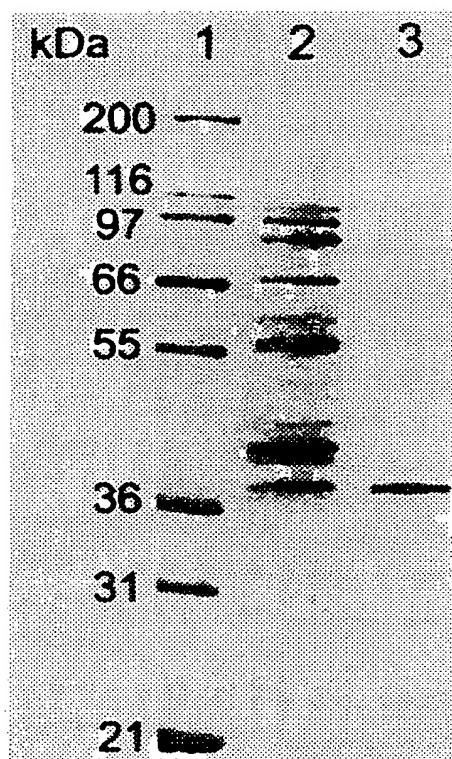
**FIG. 1**

FIG. 2B

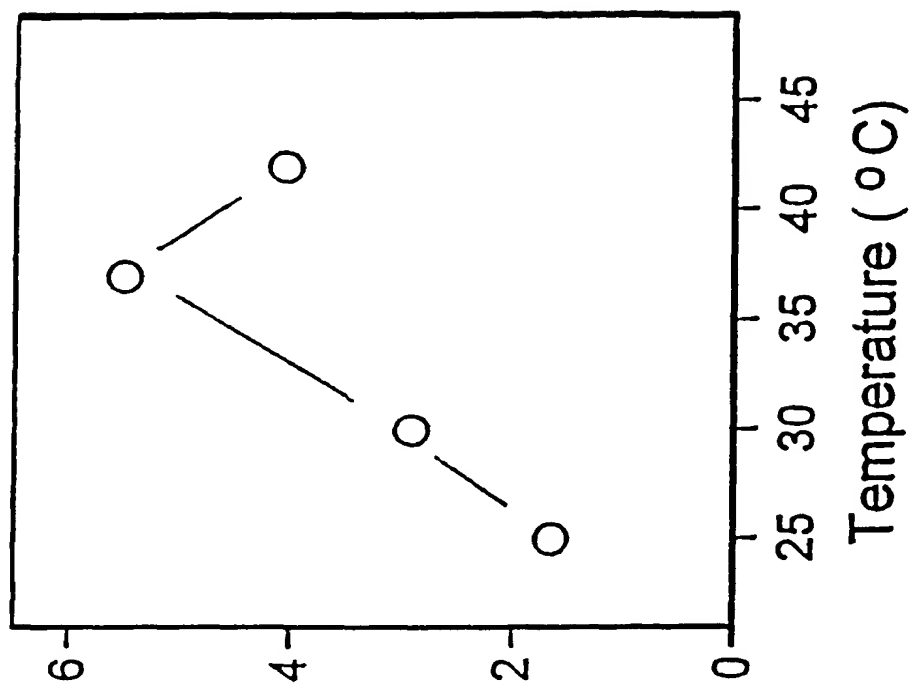


FIG. 2A

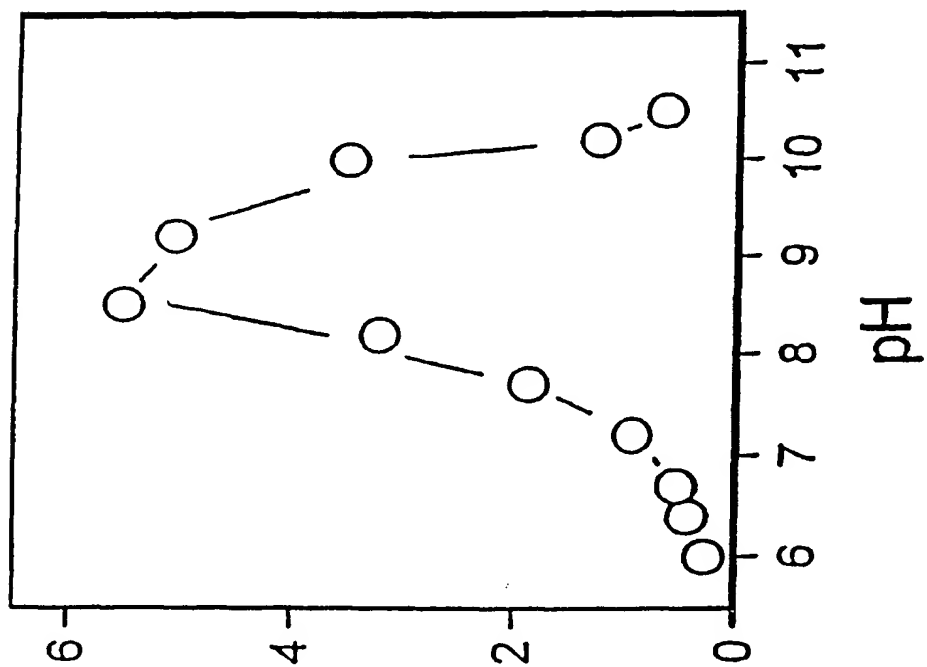
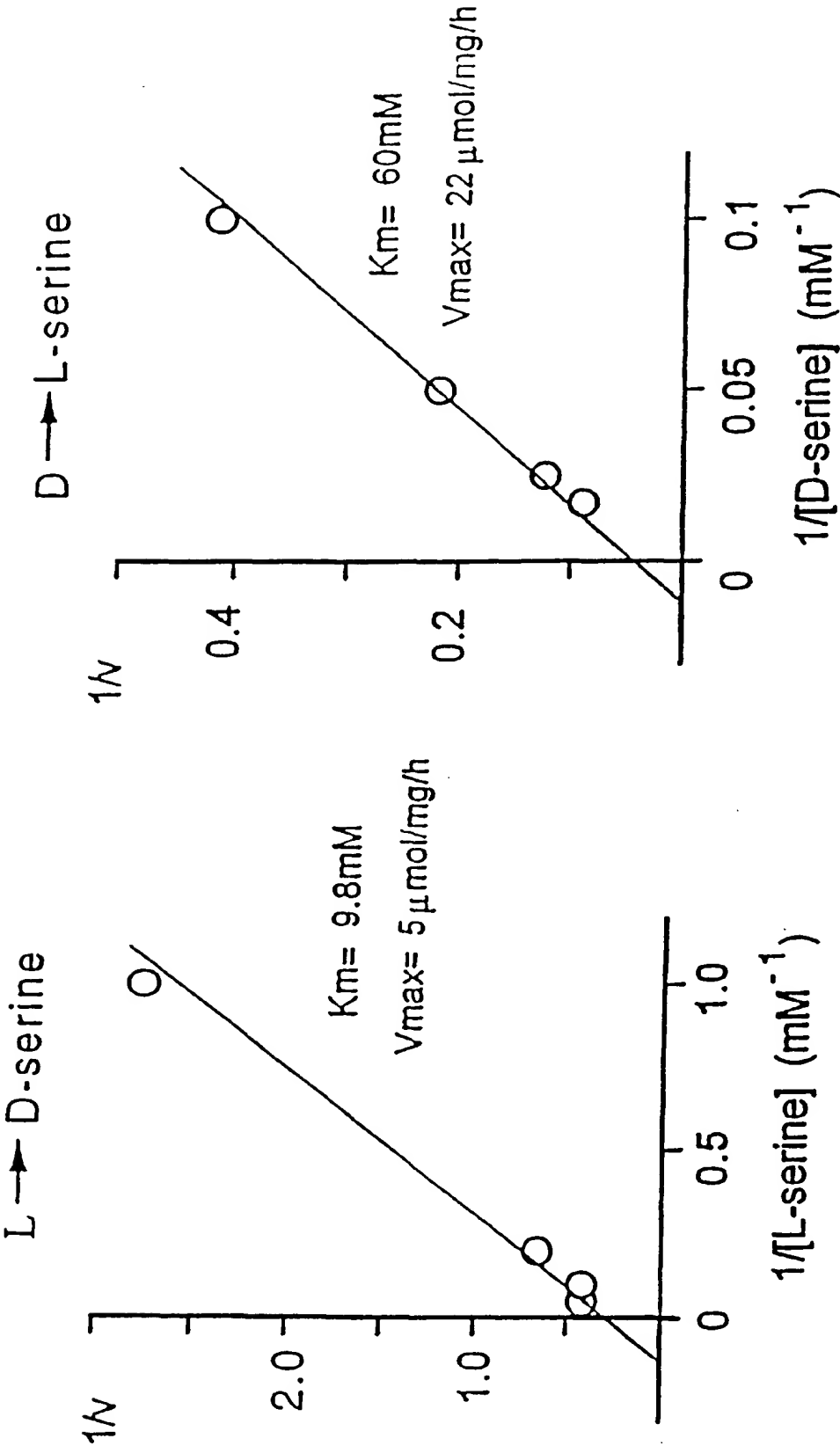
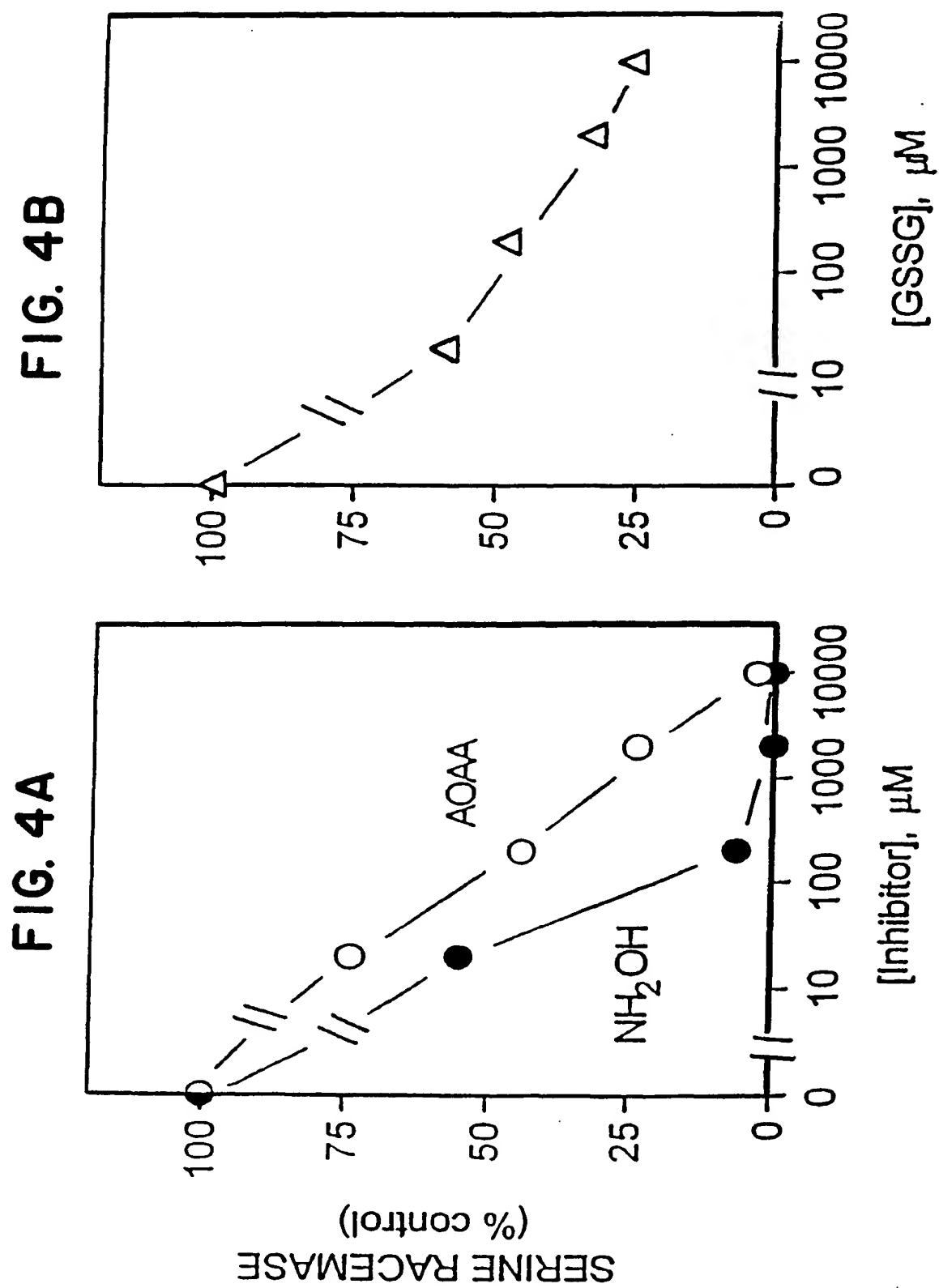
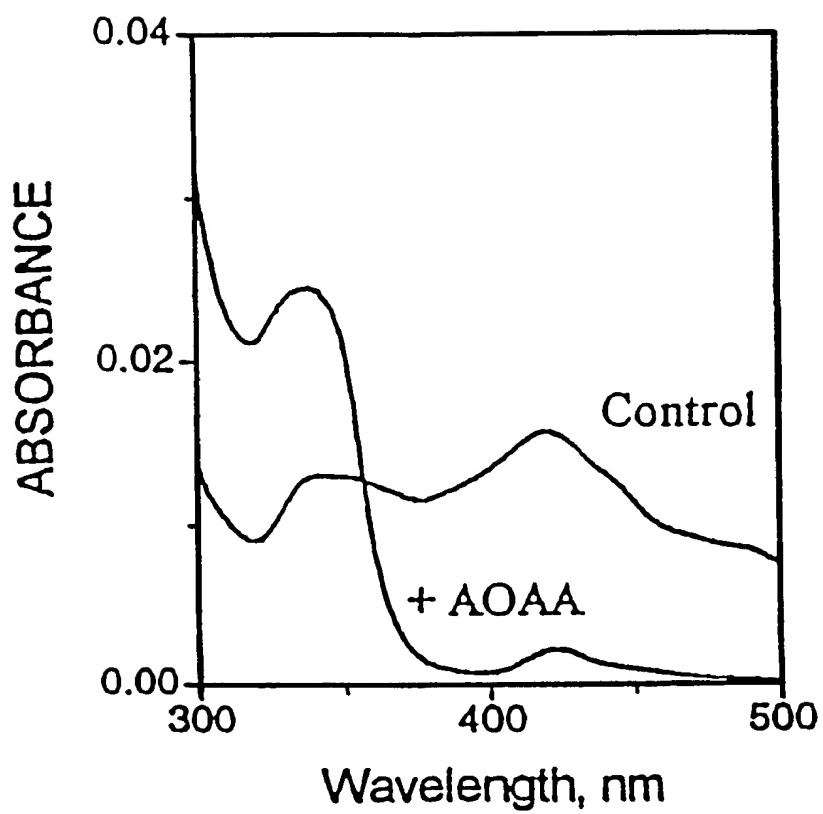


FIG. 3

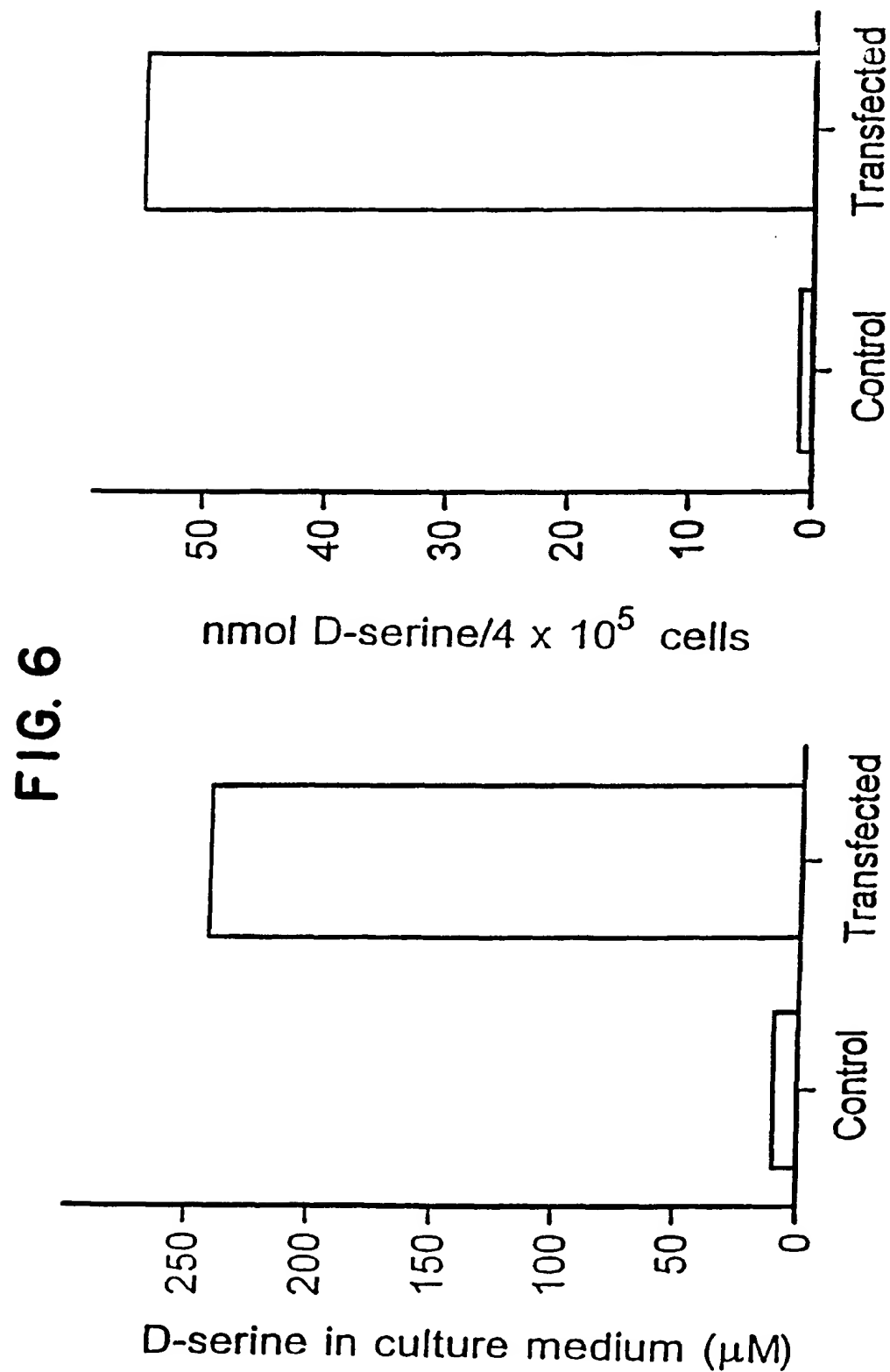


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**FIG. 5**

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**FIG. 7A**

**Mus musculus serine racemase gene, complete cds**

Source: house mouse

Organism : Mus musculus

Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;

Rodentia; Sciurognathi; Muridae; Murinae; Mus.

Strain = "Balb/c"

Dev. Stage = Adult

Tissue type = brain

Gene Starts at 219 and ends 1238

```

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## FIG. 7B

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Translation:="MCAQYCISFADVEKAHINIQDSIHLPVLTSSILNQIAGRNLFKCELFQKTGSEFKIR  
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Thr His Ser Ser Gly Asn His Gly Gln Ala Leu Thr Tyr Ala Ala Lys

85 90 95

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Cys Lys Lys Leu Ala Ile Gln Ala Tyr Gly Ala Ser Ile Val Tyr Cys

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340

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 00/00938

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/61 C12N9/90 C12N15/85 C12N5/10 C12Q1/533

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TAKUMA UO ET AL.: "Occurrence of pyridoxal 5'-phosphate-dependent Serine racemase in silkworm, Bombyx mori" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 246, no. 1, 8 May 1998 (1998-05-08), pages 31-34, XP002135965 ORLANDO, FL US abstract page 32, right-hand column, paragraph 2 -page 33, left-hand column, paragraph 3 page 34, left-hand column, last paragraph	1-13
X	GB 2 048 266 A (MITSUITOATSU CHEMICALS) 10 December 1980 (1980-12-10) page 2, line 16 - line 50 --- -/--	1-6, 9-11, 24, 28, 32-38

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

\* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

9 May 2000

Date of mailing of the international search report

23/05/2000

Name and mailing address of the ISA

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Fax: (+31-70) 340-3016

Authorized officer

Montero Lopez, B

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 00/00938

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>DAVID S. DUNLOP ET AL.: "The origin and turnover of D-Serine in brain" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 235, no. 1, 9 June 1997 (1997-06-09), pages 26-30, XP002135966 ORLANDO, FL US abstract page 30, right-hand column, paragraph 2 - paragraph 3</p> <p style="text-align: center;">---</p>	1-13
P, X	<p>HERMAN WOLOSKE ET AL.: "Serine racemase: a glial enzyme synthesizing D-Serine to regulate glutamate-N-methyl-D-aspartate neurotransmission" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 96, no. 23, 9 November 1999 (1999-11-09), pages 13409-13414, XP002135967 WASHINGTON US page 13410, left-hand column, paragraph 5 -page 13413, right-hand column, paragraph 2; figure 1</p> <p style="text-align: center;">---</p>	1-38
P, X	<p>HERMAN WOLOSKE ET AL.: "Purification of Serine racemase: Bioynthesis of the neuromodulator D-Serine" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 96, no. 2, 19 January 1999 (1999-01-19), pages 721-725, XP002135968 WASHINGTON US page 722, right-hand column, paragraph 3 -page 725, left-hand column, last paragraph</p> <p style="text-align: center;">-----</p>	1-13, 32-38

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No  
PCT/US 00/00938

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB 2048266 A	10-12-1980	JP 56134992 A	22-10-1981
		JP 1207308 C	11-05-1984
		JP 55148095 A	18-11-1980
		JP 57031438 B	05-07-1982
		JP 56005098 A	20-01-1981
		JP 61000070 B	06-01-1986
		AU 530435 B	14-07-1983
		AU 5797980 A	13-11-1980
		CA 1128443 A	27-07-1982
		CH 642947 A	15-05-1984
		DE 3017861 A	22-01-1981
		FR 2456140 A	05-12-1980
		IT 1145337 B	05-11-1986
		MX 6037 E	08-10-1984
		NL 8002603 A	11-11-1980
		US 4335209 A	15-06-1982

# PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference <b>1107.85600</b>	<b>FOR FURTHER ACTION</b>		see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. <b>PCT/US 00/ 00938</b>	International filing date (day/month/year) <b>18/01/2000</b>	(Earliest) Priority Date (day/month/year) <b>19/01/1999</b>	
Applicant <b>THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE</b>			

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

**1. Basis of the report**

a. With regard to the language, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☒ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☒ furnished subsequently to this Authority in computer readable form.

☒ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☒ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ Certain claims were found unsearchable (See Box I).

3. ☐ Unity of invention is lacking (see Box II).

4. With regard to the title,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the abstract,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is Figure No. \_\_\_\_\_

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☐ Non of the figures.

## INTERNATIONAL SEARCH REPORT

International Application No.

P US 00/00938

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/61 C12N9/90 C12N15/85 C12N5/10 C12Q1/533

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TAKUMA UO ET AL.: "Occurrence of pyridoxal 5'-phosphate-dependent Serine racemase in silkworm, Bombyx mori" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 246, no. 1, 8 May 1998 (1998-05-08), pages 31-34, XP002135965 ORLANDO, FL US abstract page 32, right-hand column, paragraph 2 -page 33, left-hand column, paragraph 3 page 34, left-hand column, last paragraph ---	1-13
X	GB 2 048 266 A (MITSUITOATSU CHEMICALS) 10 December 1980 (1980-12-10)  page 2, line 16 - line 50 --- -/--	1-6, 9-11, 24, 28, 32-38

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&amp;" document member of the same patent family

Date of the actual completion of the international search

9 May 2000

Date of mailing of the international search report

23/05/2000

Name and mailing address of the ISA

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NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Montero Lopez, B

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/00938

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DAVID S. DUNLOP ET AL.: "The origin and turnover of D-Serine in brain" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 235, no. 1, 9 June 1997 (1997-06-09), pages 26-30, XP002135966 ORLANDO, FL US abstract page 30, right-hand column, paragraph 2 - paragraph 3	1-13
P, X	HERMAN WOLOSKE ET AL.: "Serine racemase: a glial enzyme synthesizing D-Serine to regulate glutamate-N-methyl-D-aspartate neurotransmission" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 96, no. 23, 9 November 1999 (1999-11-09), pages 13409-13414, XP002135967 WASHINGTON US page 13410, left-hand column, paragraph 5 -page 13413, right-hand column, paragraph 2; figure 1	1-38
P, X	HERMAN WOLOSKE ET AL.: "Purification of Serine racemase: Bioynthesis of the neuromodulator D-Serine" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 96, no. 2, 19 January 1999 (1999-01-19), pages 721-725, XP002135968 WASHINGTON US page 722, right-hand column, paragraph 3 -page 725, left-hand column, last paragraph	1-13, 32-38

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PO US 00/00938

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB 2048266 A	10-12-1980	JP 56134992 A	22-10-1981
		JP 1207308 C	11-05-1984
		JP 55148095 A	18-11-1980
		JP 57031438 B	05-07-1982
		JP 56005098 A	20-01-1981
		JP 61000070 B	06-01-1986
		AU 530435 B	14-07-1983
		AU 5797980 A	13-11-1980
		CA 1128443 A	27-07-1982
		CH 642947 A	15-05-1984
		DE 3017861 A	22-01-1981
		FR 2456140 A	05-12-1980
		IT 1145337 B	05-11-1986
		MX 6037 E	08-10-1984
		NL 8002603 A	11-11-1980
		US 4335209 A	15-06-1982

**PCT**

REC'D 10 APR 2001

**INTERNATIONAL PRELIMINARY EXAMINATION REPORT**

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 1107.85600	<b>FOR FURTHER ACTION</b> See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/US00/00938	International filing date (day/month/year) 18/01/2000	Priority date (day/month/year) 19/01/1999
International Patent Classification (IPC) or national classification and IPC C12N15/61		
Applicant THE JOHNS HOPKINS UNIVERSITY,...		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.


2. This REPORT consists of a total of 7 sheets, including this cover sheet.

- ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☒ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand  07/08/2000	Date of completion of this report  09.04.2001
Name and mailing address of the international preliminary examining authority:   European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized officer  Montero Lopez, B  Telephone No. +31 70 340 3739



# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US00/00938

## I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).):*

### Description, pages:

1-31 as originally filed

### Claims, No.:

1-38 as originally filed

### Drawings, No.:

1-7 as originally filed

### Sequence listing part of the description, pages:

1-7, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/US00/00938

- ☐ the description,      pages:  
☐ the claims,      Nos.:  
☐ the drawings,      sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

*(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

**1. Statement**

Novelty (N)	Yes:	Claims	4-8, 12-38
	No:	Claims	1-3 9-11
Inventive step (IS)	Yes:	Claims	4-6
	No:	Claims	1-3, 7-38
Industrial applicability (IA)	Yes:	Claims	1-38
	No:	Claims	

2. Citations and explanations  
**see separate sheet**

**VII. Certain defects in the international application**

The following defects in the form or contents of the international application have been noted:  
**see separate sheet**

**VIII. Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:  
**see separate sheet**

**Re Item V**

**Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

Reference is made to the following document:

D1: Biochemical and Biophysical Research Communications, 1998, vol. 246, No. 1, pages 31-34.

1. The underlying application refers to a Serine Racemase.
2. Document D1, which is considered to represent the most relevant state of the art, discloses (cf. page 32, col. right, par. 3 - page 33, col. left, par. 1) a silkworm Serine Racemase which has a specific activity in the conversion of L- to D-Serine of 1.1 units/mg. This corresponds, according to the definition of the assay for Serine Racemase disclosed in page 32, col. right, par. 2, to 0.06  $\mu\text{mol/mg/hour}$ .
  - 2.1. In addition to the feature relating to the specific activity of the enzyme, claims 1, and 9-11 attempt to define a product, an enzyme, according to the process to obtain it (of mammalian origin). However, the method of preparation does not impart any limitation to the product. A claim directed to a product according to the process to obtain the same is therefore construed as a claim to the product as such and the features relating to the process are entirely disregarded.
  - 2.2. For the reasons specified above, the Serine Racemase disclosed in D1, showing a specific activity of 0.06  $\mu\text{mol/mg/hour}$ , is prejudicial for the novelty of claims 1-3 and 9- 11, which therefore do not comply with the requirements of Article 33(2) PCT.
3. Claims 4-6 specify the activity of the enzyme being at least 1  $\mu\text{mol L-Serine/mg/hour}$ . No such enzyme has been disclosed in the state of the art and therefore claims 4-6 are novel and comply with the requirements of Article 33(2) PCT.

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

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International application No. PCT/US00/00938

3.1. Document D1, which is considered to represent the most relevant state of the art, discloses (cf. page 32, col. right, par. 3 - page 33, col. left, par. 1) a silkworm Serine Racemase which has a specific activity in the conversion of L- to D-Serine of 0.06  $\mu\text{mol/mg/hour}$ . This activity is significantly lower than the activity of the enzymes claimed in claims 4-6. No hint exists in the state of the art which would allow the skilled person to obtain a serine racemase showing the claimed specific activity and therefore claims 4-6 are considered to involve an inventive step and to comply with the requirements of Article 33(3) PCT.

4. Claims 7, 8, 12, 13 further define the Serine Racemase by reference to its amino acid sequence. Since document D1 does not disclose the amino acid sequence of the enzyme, claims 7, 8, 12 and 13 are novel and meet the requirements of Article 33(3) PCT.

4.1. However, document D1 does specify the interest of determining the amino acid sequence of the enzyme (page 34, col. left, par. 3). The sequencing of a protein identified in the state of the art is a matter of routine for the skilled person which does not involve any inventive step. Claims 7, 8, 12 and 13, therefore do not involve an inventive step and do not comply with the requirements of Article 33(3) PCT.

5. Claims 14-31 relate to the polynucleotide encoding the Serine Racemase, as well as expression vectors and host cells comprising the same and their use for producing the enzyme. Such embodiments have not been disclosed in the state of the art and therefore claims 14-31 are novel according to Article 33(2) PCT.

5.1. However, document D1 suggests that the identification of the gene encoding the Serine Racemase may provide useful means for studying the role of D-Serine in mammalian brain (page 34, col. left, par. last). The skilled person would so be inevitably prompted to attempt the identification of the polynucleotide encoding the enzyme disclosed in D1, which he would put into practice by applying standard techniques in the art without the need of exercising any inventive step. Consequently, claims 14-31 do not involve an inventive step and do not meet the requirements of Article 33(3) PCT.

6. Claims 32-38 refer to the use of the Serine Racemase in a screening method to identify candidate therapeutic agents, not specifically disclosed in the state of the art and comply with the novelty requirements of Article 33(2) PCT.

6.1. However, such a screening process as disclosed in claims 32-38 constitutes a standard method in the art which the skilled person would apply without the need of exercising any inventive skill. Consequently, claims 32-38 do not involve an inventive step and do not meet the requirements of Article 33(3) PCT.

**Re Item VII**

**Certain defects in the international application**

1. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the document D1 is not mentioned in the description, nor is this document identified therein.

**Re Item VIII**

**Certain observations on the international application**

1. Claims 1, 13, 14 and 20 refer to "isolated" proteins and polynucleotides. Claims 14 and 20 refer as well to a "purified" polynucleotide. The applicant is kindly reminded that the degree of isolation or purification is not a technical feature of a preparation and the terms "isolated" and "purified" are therefore disregarded (Article 6 PCT).

2. Claims 1, 9-11, 13, 14, 20, 24, and 28-38 attempt to define a product, a protein, according to the process to obtain it (of mammalian, mouse, rat or human origin). However, the method of preparation does not impart any limitation to the product. A claim directed to a product according to the process to obtain the same is therefore construed as a claim to the product as such. In the present case, the product would be better defined in terms of its own structural features, such as its amino acid sequence (Article 6 PCT).

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3. Claims 1-6, 13 and 38, which define an enzyme with reference to its specific activity, do not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not clearly defined. The expression "specific activity" does not enable the skilled person to determine precisely the scope of the claim. The "specific activity" is defined in page 5 of the description, lines 21-25, with reference to Example 2 and other methods known in the art. It is therefore not clear in which conditions the specific activity is determined, which renders the scope of these claims unclear, contrary to Article 6 PCT.

4. Claims 1-6, 9-11, 14, 15, 21, 24, 28, and 32-38, 22-26, and 29 are defined merely in terms of an unclear functional feature (specific activity) and a process for preparation (mammalian origin). Such a definition renders the scope of the claims unclear and lacks the characterising features necessary for the adequate definition of the invention. The subject-matter of these claims is defined by merely stating an obviously desirable effect in view of the prior art, that is to provide a mammalian Serine Racemase (see page 34, col. left, last par. of D1). The present formulation of the claims in terms of functional features which merely state a desirable aim to be achieved and do not provide the necessary characterising features to solve the problem posed renders the scope of the claims unclear contrary to Article 6 PCT.